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<u>L4</u>	('20020149369' 'WO 2056049 A2')[ABPN1,NRPN,PN,TBAN,WKU]	3	<u>L4</u>
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L3: Entry 1 of 3

File: PGPB

Oct 17, 2002

PGPUB-DOCUMENT-NUMBER: 20020149369

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TITLE: Microfluidic device with multiple microcoil NMR detectors

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INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Peck, Tim L.	Mahomet	IL	US	
Olson, Dean	Champaign	IL	US	
Norcross, Jim	Champaign	IL	US	
Strand, David	Sherborn	MA	US	
Sweedler, Jonathan	Urbana	IL	US	

US-CL-CURRENT: 324/321; 324/306, 435/4

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
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 2. Document ID: WO 2056049 A2

L3: Entry 2 of 3

File: EPAB

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TITLE: MICROFLUIDIC DEVICE WITH MULTIPLE MICROCOIL NMR DETECTORS

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc Image										

 3. Document ID: US 20020149369 A1 WO 200256049 A2

L3: Entry 3 of 3

File: DWPI

Oct 17, 2002

DERWENT-ACC-NO: 2002-583659

DERWENT-WEEK: 200270

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TITLE: Nuclear magnetic resonance system for analysis of chemical structure of molecules, has multiple micro coil detection sites comprising respective sample management modules to which fluid sample is directed

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Term	Documents
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MICRO-COIL.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	117
MICRO-COILS.DWPI,TDBD,EPAB,JPAB,USPT,PGPB.	82
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L7: Entry 3 of 21

File: PGPB

Sep 19, 2002

DOCUMENT-IDENTIFIER: US 20020130661 A1
TITLE: Nuclear magnetic resonance analysis of multiple samples

Abstract Paragraph (1):

A Nuclear Magnetic Resonance (NMR) probe device (20) is disclosed. NMR probe device (20) includes a plurality of detection coils (30, 40) each operable to detect a signal from a corresponding one of a plurality of samples (34, 44) undergoing NMR analysis. Also included is a plurality of tuning circuits (31, 41, 38, 48) each coupled to one of detection coils (30, 40) to tune the one of the detection coils (30, 40) to a corresponding resonant frequency for the NMR analysis of the corresponding one of the samples. An electromagnetic shield (22) is positioned between a first one of the detection coils (30, 40) and a second one of the detection coils (30, 40) to isolate the first one of the detection coils (30, 40) and the second one of the detection coils (30, 40) from each other.

Summary of Invention Paragraph (2):

[0003] The present invention relates to analysis of materials based on Nuclear Magnetic Resonance (NMR), and more particularly, but not exclusively, the NMR analysis of multiple samples.

Summary of Invention Paragraph (3):

[0004] Atomic nuclei with an odd atomic mass or an odd atomic number possess a nuclear magnetic moment. NMR methods are based on the absorption and re-emission of radio frequency waves by a sample in a magnetic field that have atoms with this nuclear make-up. By way of nonlimiting example, molecules including .sup.1H, .sup.13C, .sup.19F, or 31P may be analyzed using NMR techniques to provide fast, molecule-specific qualitative and quantitative information. Such molecules exhibit resonant frequencies that are sensitive to the molecular chemical environment, making NMR a useful molecular probe. However, existing NMR equipment is generally unable to satisfactorily analyze more than one sample at a time, and correspondingly limits sample evaluation throughput. To provide for more efficient use of NMR resources, techniques to increase sample throughput would be desirable.

Summary of Invention Paragraph (5):

[0005] One form of the present invention includes a unique system to perform NMR evaluation on more than one sample at a time. Alternatively or additionally, another form of the present invention includes a unique technique to perform NMR analysis of multiple samples simultaneously.

Summary of Invention Paragraph (6):

[0006] A further form of the present invention includes NMR instrumentation comprising a probe with a plurality of coils each configured to receive a different sample. The coils each include a tuning circuit. These tuning circuits may be located external to the NMR magnet and may be electrically shielded from one another to reduce unwanted interactions.

Summary of Invention Paragraph (7):

[0007] In another form, a technique of the present invention includes providing an NMR probe with multiple coils each arranged to receive a corresponding one of a plurality of samples. The coils may each be tuned separate from the others to provide for simultaneous evaluation of the samples. The coils may each be coupled to a tuning circuit having a variable element that is adjusted to perform the tuning operation. For each tuning circuit, the coil may be coupled to the variable element by a transmission line to provide for remotely locating the variable element outside of the NMR magnetic field while the coil remains in this field. In one embodiment,

the tuning circuit includes two variable capacitor elements remotely located relative to the coil by a transmission line coupling, and another 2 fixed or variable capacitors coupled either in parallel or series, or both, in the NMR magnet before the transmission line coupling to the tuning circuit.

Summary of Invention Paragraph (8):

[0008] In yet another form, an NMR probe is provided that includes a number of coils each configured to receive a different sample. This probe may be incorporated into standard NMR equipment with only minor modifications to facilitate the simultaneous detection of multiple samples. For example, the probe may be arranged to fit into a conventional NMR magnet housing and use a common NMR transmitter amplifier for excitation of multiple samples.

Summary of Invention Paragraph (9):

[0009] Other forms of the present invention include a multicoil NMR probe arranged for operation in a magnetic field with a predetermined gradient. This gradient is used to differentiate multiple samples. The data from multiple coils in the graded field may be received by a single receiver and analyzed using one or more procedures to provide the desired differentiation. In one embodiment, a two dimensional representation of the data is created to better differentiate the samples.

Brief Description of Drawings Paragraph (2):

[0011] FIG. 1 is a partial schematic view of a NMR system of one embodiment of the present invention.

Brief Description of Drawings Paragraph (4):

[0013] FIGS. 3 and 4 are diagrams of proton coupled $\sup{13}\text{C}$ NMR spectra obtained with the system of FIG. 1 for methanol and acetone, respectively. Both spectra were acquired using a single 90 degree pulse and the same 50 kHz spectral widths. J couplings evident in the spectra are 138 Hz for methanol and 140Hz for acetone.

Brief Description of Drawings Paragraph (5):

[0014] FIG. 5 depicts $\sup{13}\text{C}$ NMR spectra for methanol and carbon tetrachloride using the system of FIG. 1.

Brief Description of Drawings Paragraph (6):

[0015] FIG. 6 is a partial schematic view of a NMR system of another embodiment of the present invention.

Brief Description of Drawings Paragraph (10):

[0019] FIG. 10 is a diagram illustrating a composite NMR spectrum of multiple H._{sub.20}/D._{sub.20} samples obtained with the probe device of FIG. 6 in a substantially homogeneous magnetic field.

Brief Description of Drawings Paragraph (11):

[0020] FIG. 11 is a diagram illustrating a composite NMR spectrum of the same FIG. 10 H._{sub.20}/D._{sub.20} samples obtained with the probe device of FIG. 6 in a graded magnetic field.

Brief Description of Drawings Paragraph (12):

[0021] FIG. 12 depicts a first composite $\sup{1}\text{H}$ NMR spectrum for 0.5 M samples of methanol, acetonitrile, t-butanol, and water in a substantially homogeneous magnetic field, and a second composite $\sup{1}\text{H}$ NMR spectrum of these samples with a magnetic field gradient applied.

Brief Description of Drawings Paragraph (14):

[0023] FIG. 14 depicts separated $\sup{1}\text{H}$ NMR spectra of each of the samples of FIG. 12.

Brief Description of Drawings Paragraph (16):

[0025] FIG. 16 depicts a composite $\sup{1}\text{H}$ NMR spectrum for samples of H._{sub.20}, methanol, t-butanol and acetonitrile (all 500 MM in D._{sub.20}) acquired with the system FIG. 6 at 300 MHz.

Brief Description of Drawings Paragraph (17):

[0026] FIG. 17 depicts a series of $\sup{1}\text{H}$ NMR spectra recorded with different Z1 shim values to provide different corresponding gradient strengths of 0 mG cm.⁻¹, 12 mG cm.⁻¹, 24 mG cm.⁻¹, and 48 mG cm.⁻¹, respectively, for the samples of FIG. 16.

Brief Description of Drawings Paragraph (28):

[0037] FIG. 31 is a partial schematic view of a NMR system of a further embodiment of the present invention.

Brief Description of Drawings Paragraph (29):

[0038] FIG. 32 is a diagram illustrating selected operating characteristics of the NMR system shown in FIGS. 31 and 33.

Brief Description of Drawings Paragraph (30):

[0039] FIG. 33 is a partial schematic view of a NMR system of yet a further embodiment of the present invention.

Brief Description of Drawings Paragraph (31):

[0040] FIG. 34 is a partial diagrammatic view of an NMR system of still a further embodiment of the present invention.

Detail Description Paragraph (3):

[0042] FIG. 1 schematically illustrates Nuclear Magnetic Resonance (NMR) system 10 of one embodiment of the present invention. Instrumentation of system 10 includes Radio Frequency (RF) transmitter 12 coupled to power splitter 13. Power splitter 13 has two outputs 13a, 13b coupled to duplexers 14a, 14b, respectively. Each duplexer 14a, 14b is operably coupled to a corresponding probe channel CH1, CH2. Duplexers 14a, 14b pass high-power level RF signals from RF transmitter 12 to probe device 20 via channels CH1, CH2.

Detail Description Paragraph (4):

[0043] Probe device 20 is removably positioned in sample space 15 of NMR magnet 16. Probe device 20 is configured to place two samples in sample space 15 for simultaneous NMR analysis. Referring additionally to FIG. 2, probe device 20 includes housing 20a that defines probe head 50 opposite base 60. Two detection coils 30, 40 are disposed within probe head 50. Coils 30, 40 are each disposed about a corresponding sample holder 30a, 40a. Sample holders 30a, 40a are each arranged to hold a different sample, and maintain the samples spatially separated from one another within probe head 50. In one embodiment, coils 30, 40 are each provided in the form of a helical winding (alternatively designated a "solenoid" configuration herein) about a glass tube which serves as the corresponding sample holder 30a, 40a. In other embodiments, coil 30 and/or coil 40 can be of a different type, including, but not limited to a saddle or bird cage coil geometry, and holders 30a, 40a can be configured for another container type and/or composition. U.S. Pat. No. 4,654,592 to Zens; U.S. Pat. No. 5,323,113 to Cory, et al.; and U.S. Pat. No. 5,929,639 to Doty provide a few nonlimiting illustrations of various types of coil geometry.

Detail Description Paragraph (5):

[0044] Probe device 20 includes channel circuitry 21 to independently couple each coil 30, 40 to a different connector 23a, 23b in base 60 in correspondence with probe channels CH1, CH2, respectively. In other embodiments, probe device 20 includes additional coils with corresponding channel circuitry and connectors to provide more than two probe channels. Accordingly, for such embodiments, more than two samples can be submitted for simultaneous NMR analysis.

Detail Description Paragraph (9):

[0048] System 10 further includes preamps 17a, 17b; receivers 18a, 18b; and reference frequency source 19. Probe channels CH1, CH2 are each electrically connected to a corresponding preamp 17a, 17b and NMR receiver 18a, 18b. Receivers 18a, 18b are electrically coupled to reference frequency source 19 in a standard manner. Fixed tuning networks 31, 41 and adjustable tuning networks 38, 48 are arranged to tune to a resonant frequency for NMR analysis of a nucleus type common to each of the samples held in coils 30, 40. During exposure to the magnetic field generated by NMR magnet 16, a suitable RF signal from transmitter 12 delivered to each coil 30, 40 excites the corresponding samples. Duplexers 14a, 14b are arranged to route the RF excitation to channels CH1, CH2 through crossed diode pair DP1 while preamps 17a, 17b are blanked to present a high input impedance. Crossed diode pair DP2 associated with each channel CH1, CH2 provides further circuit isolation and protection. Typically, RF excitation is in the form of a common 90 degree pulse; however, other interrogation technique may be alternatively or additionally be utilized as would occur to those skilled in the art.

Detail Description Paragraph (11):

[0050] Preamps 17a, 17b are activated to receive the response signals from channels CH1, CH2 via duplexers 14a, 14b for processing by NMR receivers 18a, 18b in the usual manner.

Detail Description Paragraph (14):

[0053] The experimentally observed spectra depicted in FIGS. 3 and 4 illustrate the type of data that may be acquired with system 10. For this experimental example, two coils were provided in the form of a 4 turn inductor of solenoid geometry wrapped from gauge insulated magnet wire that were each attached to a glass tube using a common epoxy adhesive. This glass tube was about 30 mm long with about a 4 millimeter (mm) outer diameter (o.d.) and about a 2 mm inner diameter (i.d.). Each sample was placed in a sealed glass tube having about a 2 mm inner diameter and a length of about 6 mm that was positioned into a corresponding one of the larger glass tubes. A nominal 11 picofarad (pf) fixed capacitor was used for each of the parallel capacitive elements 32, 42 (American Technical Ceramics Corp., Huntington Station, N.Y.). For this embodiment, a nominal 32 picofarad (pf) capacitor was utilized for each element 34, 44 between the network 31, 41 and the transmission line 36, 46, respectively; and tuning elements 38a, 38b, 48a, 48b of networks 38, 48 were provided in the form of capacitors having a nominally variable range of about 3 pf to about 11 pf (Voltronics Corporation, Denville, N.J.). The regions of the probe base containing the variable capacitors were electronically isolated from one another. It was observed that the coils did not exhibit coupling due to mutual inductance when tuned and matched to the same resonant frequency; thereby allowing different samples to be monitored using a single NMR spectrometer.

Detail Description Paragraph (15):

[0054] For FIGS. 3 and 4, spectral data was acquired at about 7.4 Tesla and both spectra were acquired at the same time in response to a single 90 degree RF pulse from the RF transmitter operating at a frequency of 75.44 MHz (corresponding to 75.440 MHz for ¹³C). In this example, the two spectra were acquired on two separate NMR receivers. The transmitted RF excitation pulse was split through the power splitter (Model ZSC-2-1W, Mini-Circuits, Brooklyn N.Y.), and each output from the power splitter was routed through crossed diodes (to reduce amplifier noise) and subsequent, independent duplexer/preamp stages. The crossed diode pairs DP1, DP2 also improved signal isolation, reducing interference during data acquisition. The receivers were phased locked using a 10 MHz reference signal from the Varian spectrometer. Simultaneous data acquisition was accomplished by using Analog-to-Digital (A/D) conversion.

Detail Description Paragraph (16):

[0055] In FIGS. 3 and 4, ¹³C NMR spectra of the two different samples each representing a single analyte compound were acquired at the same time in separate, discrete detection coils. In one coil, a sample of about 4 .mu.l of acetone, isotopically enriched to 99% at the methyl position was detected (FIG. 4). In the other coil, a sample of ¹³C enriched methanol (also 99%) of similar size was detected (FIG. 3). Enriched compounds were used to enhance the signal to noise ratio for preliminary investigations, however, the observed mass-limited sensitivity was better than that typically achieved in standard NMR probes and is in line with the sensitivity of small microcoils in general. Both spectra of FIGS. 3 and 4 were acquired with the same 50 kHz spectral widths. J couplings evident in the spectra are 138 Hz for methanol and 140 Hz for acetone.

Detail Description Paragraph (18):

[0057] In an alternative embodiment of system 10, a single receiver may be utilized that is switched between channels CH1 and CH2 to acquire data. Correspondingly, a single computer can be used to operate the spectrometer and to acquire the signals from different samples by using a multichannel analog to digital (A/D) converter. The selected frequencies of the transmitter and receiver may vary according the particular application as would occur to those skilled in the art. Further, the RF transmitter, duplexers, receivers, and associated connections of system 10 may otherwise be arranged as would occur to those skilled in the art without departing from the spirit of the present invention. In other embodiments, other coil types and geometries may be utilized, and/or the coils may be differently sized in alternative embodiments, including the microcoil variety. As used herein, a "microcoil" has a maximum diameter of no more than about 1 millimeter (mm). In still other embodiments, more than two samples may be evaluated simultaneously in the same NMR probe by adding coils suitably decoupled by appropriate ground planes, shielding, and/or coil orientations; with corresponding tuning circuitry, duplexers, associated

connections, and (optionally) receivers in accordance with the teachings of the present invention. Alternatively or additionally, RF excitation may be provided to each sample from a different source than the detection coil, such as a dedicated excitation coil.

Detail Description Paragraph (20):

[0059] For system 110, multiple samples are differentiated by applying a magnetic field gradient. When a magnetic field with a gradient component is applied, samples in different regions of space experience different magnetic fields. A spatially dependent frequency offset is introduced by the magnetic field gradient that is unique to each properly positioned sample. The application of field gradients allows for the signals from multiple samples to be detected using only a single receiver. Accordingly, differentiation of spectra for multiple samples can be determined from a two dimensional representation, with the first dimension providing the spatial information and the second dimension providing spectral information. By combining this technology with NMR microcoils, a substantial number of samples, limited only by the usable region of the NMR magnetic field, can be simultaneously analyzed.

Detail Description Paragraph (21):

[0060] System 110 includes NMR spectrometer instrumentation 111 operatively coupled to processor 119 and removable probe device 120. As depicted in FIG. 6, probe device 120 is disposed in sample space 115 of NMR magnetic field source device 116. Furthermore, probe device 120 is coupled to sample control instrumentation 122.

Detail Description Paragraph (22):

[0061] NMR spectrometer instrumentation 111 includes a controllable RF transmitter 112 and NMR receiver 118 commonly coupled to probe device 120 by probe channel PC1. NMR spectrometer instrumentation 111 also includes controller 113 to control the operations of RF transmitter 112 and receiver 118 and to provide an interface with processor 119.

Detail Description Paragraph (23):

[0062] processor 119 and the constituents of instrumentation 111 (such as transmitter 112, controller 113, and/or receiver 118) may be comprised of one or more components integrated to automatically process a number of samples. Alternatively, one or more components of system 110 may be remotely located relative to the others, and/or may be configured to optionally provide remote control of NMR processing with system 110. In one embodiment, processor 119 is in the form of a desktop Personal Computer (PC) programmed to perform the various indicated operations, and includes various input devices, such as a keyboard and/or mouse; and various output devices, such as a graphic display, printer, and/or plotter. For this embodiment, instrumentation 111 is in the form of a standard NMR spectrometer that provides spectral data to processor 119 by portable disk and/or a hardwired interface.

Detail Description Paragraph (27):

[0066] Referring also to FIG. 8, sample holders 132 are mounted in U-shaped yoke 152 connected to probe base 160. The opposing ends of sample holders 132 are each in fluid communication with a pair of sample conduits 136a, 136b (collectively designated sample conduits 136) that are connected to sample control instrumentation 122. In cooperation with sample control instrumentation 122, sample holders 132a, 132b, 132c, 132d are each arranged to selectively receive a corresponding fluent sample 134a, 134b, 134c, 134d (collectively designated samples 134). Samples 134 may be changed from time-to-time through the corresponding pair of conduits 136a, 136b without removing probe device 120 from sample space 115. Accordingly, the degree of likelihood that adjustments will need to be made between sample interrogations is reduced, potentially increasing throughput. Also, a higher throughput may be realized compared to standard NMR equipment that only tests one sample at a time. This sample delivery and exchange arrangement is also suitable for performing various complex molecular analysis techniques, including but not limited to Capillary Electrophoresis (CE), and Liquid Chromatography (LC) NMR (LC-NMR).

Detail Description Paragraph (29):

[0068] Tuning network 140 provides for tuning to a resonance frequency appropriate for NMR interrogation of samples 134 and is located in adjacent probe base 160 at the bottom of yoke 152. Probe base 160 is threaded to receive a threaded housing 150. When threaded together, probe base 160 and housing 150 define fluid chamber 166. Chamber 166 is arranged to optionally receive and contain a susceptibility matching fluid to further improve resolution of spectra detected with coils 130.

Detail Description Paragraph (30):

[0069] The flow chart of FIG. 9 depicts representative stages of process 210 to perform NMR analysis of samples 134 with system 110. Process 210 starts with stage 212. In stage 212, sample control instrumentation 122 is utilized to load samples 134 into holders 132 via conduits 136a, and flush-out any previous samples via conduits 136b. After samples 134 are loaded into holders 132, a generally homogeneous magnetic field is applied in sample space 115 with magnetic source device 116 and samples 134 are excited with an appropriate RF signal from transmitter 112 via coils 130 in stage 214. During stage 214, the four regions corresponding to coils 130, holders 132, and samples 134 experience generally the same magnetic field, B.sub.o. Process 210 continues with stage 216. In stage 216, the collective response of samples 134 to the RF excitation signals are detected with coils 130 and transmitted as response signals by coils 130 to receiver 118 for analysis. The corresponding spectral data S.sub.0 of samples 134 is determined from the coil response signals and stored by instrumentation 111 and/or processor 119. Referring additionally to FIG. 10, a composite spectrum for an identical sample 134 of H.sub.2O/D.sub.2O in each coil 132 of probe device 120 is illustrated. This composite spectrum has a single peak corresponding to a single resonance in the homogenous field.

Detail Description Paragraph (36):

[0075] Referring to FIG. 12, an experimental NMR .sup.1H spectrum 230a is illustrated for 0.50 M samples of water (.delta.=4.7 ppm), acetonitrile (2.0 ppm), methanol (3.3 and 4.7 ppm) and t-butanol (1.2 and 4.76 ppm) in D.sub.2O in separate coils of a four-coil probe. The spectrum of FIG. 12 was obtained without a predetermined magnetic field gradient in sample space 115 to correspond to spectrum S.sub.0 obtained in stage 216. FIG. 12 also includes a frequency-shifted spectrum 230b for the same sample set as used to provide spectrum 230a. For spectrum 230b, an applied gradient of about 48 mG/cm was provided to correspond to spectrum S.sub.G obtained in stage 220. Each of the peaks in spectrum 230b shifts a different amount or in a different direction according to the position of each individual coil in the applied field gradient. The OH region is split into four lines due to contributions to this peak from each of the coils.

Detail Description Paragraph (44):

[0082] Experimental verification of procedure 310 was conducted. For this experimental example, four coils were wrapped from a high purity polyurethane coated 36-gauge copper wire (California Fine Wire Co. Grover Beach, Calif.) around fused silica capillaries (about 20 mm long, about a 1.6 mm outer diameter, about a 0.8 mm inner diameter) which served as both the coil form and sample holder. The inductance of each coil was approximately 20 nanohenries (nH). The coils were attached to the capillary tubes using a cyanoacrylate adhesive (Krazy Glue, Borden Inc. Columbus, Ohio). The coils were configured with four (4) turns each having an inner diameter of about 1.6 mm and a length of about 1.0 mm. The sample tubes were mounted in a PVC coil holder (corresponding to yoke 152) that held the capillary tubes with an intercoil spacing (center to center) of about 3.2 mm. The entire coil array was housed in a removable PVC container that was filled with Fluorinert FC-43 (Syn Quest Laboratories, Alachua, Fla.), a susceptibility matching fluid that has been shown to improve magnetic field homogeneity by minimizing field distortions induced by copper NMR coils. The PVC container was threaded and employed o-ring seals to prevent leakage of the Fluorinert fluid. The coil leads were connected in parallel and cut to the same length so that the resistance and inductance of each of the coils were similar. A single resonant circuit was constructed using the four parallel coils and non-magnetic tunable capacitors (Voltronics, Denville, N.J.) to tune and match the circuit. The variable capacitors were located directly beneath the sample region. With all four coils in parallel the circuit has a tuning range of about .2 MHz centered around 300 MHz and a resonant Q of about 60. The coil housing was mounted atop a narrow-bore (about 39 mm diameter) probe body and used a semi-rigid copper coaxial line to connect the resonant circuit at the top of the probe to a BNC connector at the base. To allow flow introduction of samples, teflon tubes (about 2.0 mm outer diameter, Small Parts Inc., Miami Shores, Fla.) were connected to the capillaries using polyolefin heat-shrink (Small Parts Inc., Miami Shores, Fla.) and sealed with Torr-Seal (Varian Associates, Palo Alto, Calif.). Samples were loaded using a syringe.

Detail Description Paragraph (45):

[0083] The probe was centered in an NMR magnet by loading four H.sub.2O/D.sub.2O samples and adjusting the linear gradient (Z1 shim) to separate the peaks from the

individual coils. The center was chosen as the point at which the top and bottom coils were shifted in frequency by an equal and opposite amount. In order to identify the NMR spectrum as originating from a particular sample volume, the external reference procedure was utilized for this experiment. Gradient field adjustments were made by changing a Z1 shim power supply control for a Z-directional gradient coil of the NMR magnet. The nongraded composite spectrum of the samples was acquired with the Z1 shim set at its optimum value, and a second spectrum was subsequently acquired with Z1 set to a value which results in a predetermined shift for each sample coil. This shift was calibrated beforehand and remained relatively constant for a given coil configuration.

Detail Description Paragraph (46):

[0084] Spectra were collected using a Varian Unity-Plus spectrometer operating at 300 MHz for .sup.1H. Typically, one or four transients (with Cyclops receiver phase cycling and a recycle delay of 5 s) were accumulated for each experiment. A composite .sup.1H NMR spectrum is shown in FIG. 16 for 500 mM samples of H.sub.20 (.delta.=4.7 ppm), methanol (3.2 and 4.7 ppm), acetonitrile (1.9 ppm), and t-butanol (1.1 and 4.7 ppm) in D.sub.20 that had been loaded into separate coils of the flowing sample four-coil probe device. The line widths (FWHM) are, respectively, 3.1 Hz, 2.8 Hz, 3.6 Hz, and 3.4 Hz. Typical 90.degree. pulse times were 6 .mu.s using a transmitter power of roughly 1 W. The measured mass sensitivity, S.sub.m, (defined as S/N per .mu.mol of analyte) was 4200 for the t-butanol peak after 1 acquisition, using an apodization of 3 Hz (S.sub.m=2700 for an apodization of 1 Hz). Pulse calibration data showed that the four coils have similar 90.degree. pulse lengths and RF field homogeneity. It should be appreciated that the FIG. 12 and FIG. 16 spectra were based on the same samples. The peaks of the FIG. 16 spectrum differed slightly from the peaks of the FIG. 12 spectrum due to typical variations in equipment and field settings.

Detail Description Paragraph (47):

[0085] As discussed above, upon the application of a linear field gradient, the NMR spectrum of each analyte is shifted by a value dependent on its location in a particular sample coil. The strength of the gradient was adjusted by changing the value of the first-order axial (Z1) shim setting by increments of 1000 (out of a possible +-32,000), corresponding to an increase of the field gradient strength of roughly 12 mG cm.sup.-1 per increment. FIG. 17 shows a series of .sup.1H spectra obtained with the coils loaded with same four samples as for FIG. 16, but now recorded with different values of the applied field gradient. In FIG. 17, spectrum 400a is the same as the spectrum of FIG. 16, with the shim value set to 0 mG cm.sup.-1. Spectrum 400b of FIG. 17 is provided with the shim value set to generate a gradient of 12 mG cm.sup.-1. Also in FIG. 17, spectrum 400c is provided with the shim value set to generate a gradient of 24 mG cm.sup.-1, and spectrum 400d is provided with the shim value set to generate a gradient of 48 mG cm.sup.-1.

Detail Description Paragraph (54):

[0092] Still another technique to differentiate a composite NMR spectrum of different samples is to apply reference deconvolution. Reference deconvolution is performed by multiplying the experimental time-domain data (Free Induction Decay data or "FID") by the complex ratio "R" of an ideal FID and a reference FID. This procedure can also be incorporated into programming for processor 119. The reference FID is constructed from the experimental spectrum by zeroing all parts of the experimental spectrum except those containing the well-resolved reference signal and taking its inverse Fourier transform. The ideal FID is similarly generated by placing a single point (delta function) at the peak of the reference signal and zeroing the rest of the spectrum, followed by inverse Fourier transformation. The corrected experimental FID is calculated by multiplying the experimental FID by R, and Fourier transformation yields the corrected FID.

Detail Description Paragraph (57):

[0095] To improve the separation of more complex sample spectra from a composite spectrum representative of multiple samples, multidimensional NMR techniques may be incorporated into stage 230 of process 210 (FIG. 9) in other embodiments of the present invention, including, but not limited to two-dimensional (2D) Correlated Spectroscopy (COSY). By spreading out the resonances into two or more dimensions, highly dense one dimensional (1D) spectra can be considerably simplified. In correspondence with one experimental example, FIG. 25 shows the COSY spectrum of 0.50 M 1-propanol, dichloroacetic acid, ethanol, and acetaldehyde in D.sub.20 each loaded into a different sample coil. This composite spectrum was acquired with a substantially homogeneous magnetic field in correspondence with stage 214 of process

210. The spectrum shows a number of well resolved peaks, including five cross peaks. Several of the OH peaks are missing because they have exchanged with the deuterated solvent.

Detail Description Paragraph (61):

[0098] Due to the high density of peaks along the diagonal in the 2D COSY spectra, there may be significant overlapping of peaks between the back-shifted and the original spectra that gives rise to spurious peaks along the sub-spectra diagonal. This high level of congestion along the diagonal in 2D spectra would make it difficult to rely on these peaks for coil assignment. However, by instead using the cross peaks, advantage may be taken of the generally higher resolution inherent in this type of multidimensional NMR. The diagonal peaks of S.sup.i are suppressed in accordance with equation (4) as follows:

Detail Description Paragraph (64):

[0100] One alternative method is to apply pulsed field gradients during a portion of the NMR experiment. For example, a small pulsed field gradient applied during the acquisition time (t_2) of the COSY experiment will result in a 2D spectrum in which the separate samples are shifted in frequency along a single frequency axis, in this case F_2 . Alternatively, large pulsed field gradients can be used to generate subspectra in a different manner. A large pulse field gradient will shift the sample spectra into completely different frequency ranges such that individual spectra corresponding to the individual samples can be obtained directly. This is accomplished by the application of a large pulsed field gradient in conjunction with frequency selective RF excitation pulse that excites only a single sample by taking advantage of the unique frequency shift provided by the applied field gradient.

Detail Description Paragraph (69):

[0105] Processes 210, 610 and procedures 310, 410, 510 described in connection with system 110 may be combined, substituted, rearranged, reordered, deleted and altered as would occur to those skilled in the art for an application of interest. Moreover, for process 210, the first magnetic field $B_{\text{sub}0}$ provided in stage 214 need not be substantially homogeneous. Instead, in other embodiments, B_0 may be a known gradient difference relative to the field applied in stage 218 that is not homogeneous through sample space 115. Likewise, system 110 and one or more of these processes and procedures may be combined with one or more isolated tuning network/coil combinations described for system 10. In still other embodiments, the sampling parallelism of system may be generally increased as a function of the size of the homogeneous region of the NMR magnet. In one example, for a 7.05 T wide-bore magnet, this region extends over 20 mm. In an example having microcoils capable of acquiring high-resolution data with outer diameters on the order of 350 micrometers (.mu.m), and accounting for any broadening upon application of the gradient, the minimum coil spacing should be on the order of the coil diameter. These parameters allow for at least 10 microcoils to be located in the 20 mm region of the magnet. Such a probe would provide for a corresponding 10-fold reduction in throughput, which in conjunction with flow-through samples, would represent a significant advance in high-throughput screening compared to conventional single sample NMR techniques.

Detail Description Paragraph (70):

[0106] FIG. 31 provides a schematic view of NMR system 710 of another embodiment of the present invention. System 710 includes NMR instrumentation 711 with RF excitation transmitter (TXR) 712 and NMR receiver (RXR) 718. Receiver 718 is coupled to probe network 731 by transmission line 736. Network 731 includes coils 730a, 730b each disposed about sample holder 732a, 732b, respectively. Each sample holder 732a, 732b is depicted as the "flow-through" type previously described and is configured to receive a corresponding sample 734a, 734b. Coils 730a, 730b are each of a solenoid configuration wound about a corresponding centerline axis 733a, 733b. Coils 730a, 730b are oriented so that centerline axes 733a, 733b are approximately parallel.

Detail Description Paragraph (71):

[0107] RF transmitter 712 of instrumentation 711 is coupled to a separate excitation coil 740 to provide an RF stimulus signal to samples 734a, 734b. Coil 740 is wound and centered relative to an axis that is generally perpendicular to the view plane of FIG. 31 and axes 733a, 733b. Probe network 731 and excitation coil 740 are arranged to be placed in a magnetic field suitable to perform NMR analysis. For the described orientation of coils 730a, 730b, 740; samples 734a, 734b will be excited generally in-phase with one another by a suitable RF signal from coil 740, as designated by the common direction of the arrows in sample holders 732a, 732b.

Detail Description Paragraph (74):

[0110] Referring to FIG. 33, system 810 of another embodiment of the present invention is illustrated. System 810 includes a transmitting network comprised of NMR transmitter 812 to selectively provide an RF excitation signal, a matching network 813, and a crossed diode pair DPI. System 810 also includes probe network 831 arranged for placement in a magnetic field suitable to perform NMR analysis. A receiving network 841 of system 810 includes crossed diode pair DP2 and transmission line 836 that are electrically coupled to probe network 831. Receiving network 841 also includes matching network and NMR receiver 818 that are electrically coupled to transmission line 836 opposite probe network 831.

Detail Description Paragraph (76):

[0112] As explained in connection with FIG. 32, samples 834a, 834b can be selected to reduce the resulting spectral response of constituents common to both samples. Occasionally, a solvent suitable to dissolve an NMR analyte of interest provides undesirable spectral contributions. Advantageously, the ability to attenuate or cancel the spectral contribution of a substance common to both samples for the "antiparallel" coil configurations of systems 710, 810 provides a means to suppress solvent response in a solvent/analyte mixture. As in the case of the previously described systems 10, 110; systems 710, 810 may be adapted to use coils of different geometries. Also, the arrangements of systems 710, 810 may be combined with the probe circuitry of system 10 and/or 110 as would occur to those skilled in the art.

Detail Description Paragraph (77):

[0113] FIG. 34 depicts NMR system 910 of still another embodiment of the present invention. System 910 includes NMR instrumentation 111 with NMR RF transmitter (TXR) 112, controller 113, and NMR receiver (RXR) 118 of the type previously described in connection with system 110 of FIG. 6. Likewise, system 910 includes processor 119 coupled to instrumentation 111 in the manner previously described for system 110. Instrumentation 111 is electrically coupled to probe device 920 by transmission line 936. Probe device 920 is shown in partial section and is disposed in sample space 115 of NMR magnetic field source 116 also previously described in connection with system 110. System 910 may further include sample control instrumentation of the type provided in system 110 (not shown).

Detail Description Paragraph (79):

[0115] Each circuit board 922 further includes an NMR detection coil 930a, 930b, 930c (collectively designated coils 930), respectively. Coils 930 are each arranged to be disposed about a different sample submitted for NMR analysis analogous to the manner described for samples 134 of system 110; however, coils 930 are each mounted to its respective circuit board 922. Circuit boards 922 further include trimming/tuning components, a few of which are designated by reference numeral 935. Components 935 have selected electrical connections with coils 930 and can be configured to adjust for small differences in coil inductance or resistance. Accordingly, components 935 are commonly in the form of resistors and/or capacitors, but may alternatively or additionally include other types as would occur to those skilled in the art.

Detail Description Paragraph (83):

[0119] In general, the various embodiments of the present invention provide corresponding techniques to simply and cost-effectively increase NMR sample throughput. In embodiments including the investigation of Structure Activity Relationships (SAR) by NMR, one is interested in identifying only the molecules that interact strongly with large proteins. These molecules typically have significantly shorter relaxation times than other, non-interacting molecules. Therefore, by using spectral editing methods to discriminate against the non-interacting molecules, parallel sample coils of the present invention may be used to advantage. In other embodiments, probes are utilized for process monitoring and/or control. Furthermore, the deviations of NMR spectrums from a known standard spectrum may be monitored according to the present invention to identify potential problems. Moreover, the present invention includes embodiments having multi-coil probes for monitoring multiple reactions or processes in parallel.

Detail Description Paragraph (84):

[0120] Other embodiments of the present inventions include applications to a broad range of problems in analytical chemistry. For example, there is a growing need for the rapid analysis of large numbers of compounds in the pharmaceutical industry to identify potential drug candidates. In this area, Heteronuclear Multiple Quantum

Coherence (HMQC) techniques can be applied to investigate SAR. S. B. Shuker, P. J. Hajduk, R. P. Meadows, S. W. Fesik, Science 274 (1996) 1531; and P. J. Hajduk, E. T. Olejniczak, S. W. Fesik, Journal of the American Chemical Society 119 (1997) 12257 are cited as sources of additional information concerning HMQC techniques. Parallel NMR analysis will be advantageous in such an application. In another embodiment, for combinatorial chemistry, where large numbers of somewhat similar compounds are quickly synthesized, a rapid NMR analytical method could be desirable. Also, embodiments of the present inventions include coupling the structural determination capabilities of NMR with chromatographic separation techniques such as LC-NMR and CE. Other variations and embodiments of the present invention include utilizing the unique NMR probe designs of the present invention with microcoils, flow-through probes, nano-volume probes and solvent suppression pulse sequences--such embodiments may also include combinatorial synthetic methods and methods to analyze large molecular libraries.

Detail Description Paragraph (86):

[0122] All references to experiments and results are intended to be illustrative of the present invention and should not be considered limiting or restrictive with regard to the scope of the present invention. Further, any theory of operation, proof, or finding stated herein is meant to further enhance understanding of the present invention and is not intended to make the scope of the present invention dependent upon such theory, proof, or finding. All publications, patents, and patent applications cited in this specification are herein incorporated by reference as if each individual publication, patent, or patent application were specifically and individually indicated to be incorporated by reference and set forth in its entirety herein. Documents to be incorporated by reference include, but are not limited to: (1) U.S. Provisional Patent Application No. 60/121,869, filed Feb. 26, 1999; (2) Hou, T.; MacNamara, E.; Raftery, D. *Analytica Chimica Acta* 400 (1999) 297; (3) Fisher, G.; Williams, S.; Raftery, D. *Analytica Chimica Acta* 397 (1999) 9-16 and (4) Fisher, G.; Pettuci, C.; Raftery D. *Journal of Magnetic Resonance*, 138 (1999) 160-163. While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiment has been shown and described and that all changes, equivalents, and modifications that come within the spirit of the invention are desired to be protected.

CLAIMS:

1. An apparatus, comprising: an NMR transmitter to analyze a number of samples; a number of sample holders each operable to receive a corresponding one of the samples for NMR analysis; a plurality of detection coils each operable to detect a response of the corresponding one of the samples to one or more signals from said NMR transmitter; an adjustable magnetic field source proximate to a sample space arranged to receive said sample holders, said magnetic field source being operable to selectively provide: a first magnetic field to generate a first response of the samples when received in said sample space, a second magnetic field having a gradient relative to said first magnetic field to generate a second response of the samples when received in said sample space, said second response corresponding to a number of frequency shifts relative to said first response, said frequency shifts each corresponding to a different one of the samples; and a processor operable to determine a number of spectral characterizations as a function of said first sample response and said second response, the spectral characterizations each being representative of a different one of the samples.

4. The apparatus of claim 1, wherein said processor is operable to perform multidimensional NMR analysis.

7. The apparatus of claim 1, further comprising an NMR receiver coupled to said detection coils and a control to selectively adjust the gradient.

10. An apparatus, comprising: an NMR transmitter; an magnet device operable to provide a magnetic field for NMR analysis; a number of sample holders each operable to expose a corresponding one of a number of samples to the magnetic field; a plurality of detection coils each operable to detect a response of the corresponding one of the samples to one or more signals from said NMR transmitter; a number of tuning circuits each coupled to a different one of said detection coils to tune said different one of said detection coils to a resonant frequency for the corresponding one of the samples; and at least one receiver coupled to said detection coils.

16. The apparatus of any of claims 1-15, wherein: said detection coils are each of a microcoil variety with a diameter of less than about 1 millimeter; and said sample holders each include a tube disposed within a corresponding one of said detection coils.

18. An apparatus, comprising: NMR probe device, said NMR probe device including: a number of detection coils each operable to detect a signal from a corresponding one of a plurality of samples undergoing NMR analysis; a plurality of tuning circuits each coupled to a different one of said detection coils to tune said different one of said detection coils to a corresponding resonant frequency for the NMR analysis of the corresponding one of the samples; and an electromagnetic shield positioned between a first one of said detection coils and a second one of said detection coils to isolate said first one of said detection coils and said second one of said detection coils from each other.

20. An apparatus, comprising: an NMR probe device, said NMR probe device including: a plurality of sample holders each operable to expose a different one of a plurality of samples to a magnetic field for NMR analysis; a plurality of detection coils each operable to selectively detect a response from a corresponding one of the samples; and a ground plane positioned between a first one of said detection coils and a second one of said detection coils to decouple said first one of said coils and said second one of said coils from each other.

22. An apparatus, comprising: an NMR probe device to perform NMR analysis on a plurality of samples, said NMR probe device including: a plurality of sample holders each operable to receive a different one of the samples; a plurality of detection coils each operable to detect a signal from a corresponding one of the samples undergoing the NMR analysis; a plurality of first tuning networks each coupled to a different one of said detection coils; a plurality of second tuning networks each including at least one adjustable element; and a plurality of transmission lines each electrically coupled between a respective one of said first tuning networks and said second tuning networks.

26. The apparatus of any of claims 18-25, further comprising: an NMR transmitter to provide an RF signal to excite the samples; a magnetic field source proximate to a sample space configured to receive said probe device; and at least one NMR receiver.

30. The apparatus of any of claims 18-25, wherein said detection coils are each of a microcoil variety with a diameter of less than about 1 millimeter and said detection coils are each of a solenoid or saddle configuration.

33. A method, comprising: operating an NMR spectroscopy system including a sample space and a plurality of detection coils each disposed about a corresponding one of a plurality of separated samples in the sample space; generating a first magnetic field in the sample space; detecting a first response of the samples during generation of the first magnetic field; generating a second magnetic field in the sample space, the second magnetic field including a gradient relative to the first magnetic field; detecting a second response from the samples during generation of the second magnetic field; and determining a plurality of NMR spectra each corresponding to a different one of the samples in accordance with the first response and the second response.

34. The method of claim 33, further comprising performing a multidimensional NMR analysis of the samples.

37. A method, comprising: generating a magnetic field with a predetermined gradient in a sample space containing a plurality of detection coils each disposed about a corresponding one of a number of samples; detecting a number of sample responses each corresponding to excitation of a respective one of the samples by a different excitation frequency during generation of the magnetic field; and determining a number of NMR spectral characterizations from the sample responses, the NMR spectral characterizations each being representative of a different one of the samples.

43. The method of any of claims 33-39, wherein said detection coils number at least four and are each of a microcoil variety with a diameter of less than about 1 millimeter.

45. A method, comprising: providing an NMR spectroscopy system including a magnetic

field source proximate to a sample space; positioning a plurality of sample holders in the sample cavity, the sample holders each carrying a different one of a plurality of detection coils; orienting a first one of the detection coils relative to a second one of the detection coils to minimize cross-talk between the first one of the detection coils and the second one of the detection coils based on geometry of the first one of the detection coils and the second one of the detection coils; and operating the **NMR** spectroscopy system to analyze a plurality of different samples each disposed in a corresponding one of the sample holders.

52. The method of any of claims 45-48, wherein the **NMR** spectroscopy system includes: an **NMR** transmitter to provide an RF signal to excite the samples; a magnetic field source proximate to a sample space configured to receive said probe device; and at least one **NMR** receiver.

54. The method of any of claims 45-48, wherein the detection coils are each of a microcoil variety with a diameter of less than about 1 millimeter.

55. An apparatus, comprising: an **NMR** probe device, including: an RF excitation source; a first coil operable to be disposed about a first sample to detect a first sample response to an excitation signal from said RF excitation source; a second coil operable to be disposed about a second sample to detect a second sample response to the excitation signal, said second coil being connected in series with said first coil; and wherein said first coil and said second coil are disposed relative to said RF excitation source to provide a phase difference between the first sample response and the second sample response to at least partially cancel one or more response components corresponding to a common material in the first sample and the second sample.

61. The apparatus of any of claims 55-58, wherein said RF excitation source includes and **NMR** transmitter coupled to said first coil and said second coil to excite the first sample and the second sample in parallel, and further comprising: a magnetic field source proximate to a sample space configured to receive said probe device; and at least one **NMR** receiver coupled to said first coil and said second coil.

62. The apparatus of any of claims 55-58, wherein said first coil and said second coil are each of a microcoil variety with a diameter of less than about 1 millimeter.

63. A method, comprising: preparing a first sample comprised of a solvent and a second sample comprised of a mixture of the solvent and an analyte; exciting the first sample and the second sample to perform **NMR** analysis of the analyte; and detecting a first sample response with a first coil and a second sample response with a second coil, the first sample response including a phase difference relative to the second sample response to reduce solvent interference with the **NMR** analysis of the analyte.

68. The method of any of claims 63-66, wherein the first coil and the second coil are each of a microcoil variety with a diameter of less than about 1 millimeter.

L7: Entry 4 of 21

File: PGPB

Aug 8, 2002

DOCUMENT-IDENTIFIER: US 20020105327 A1
TITLE: Steep solvent gradient NMR analysis method

Abstract Paragraph (1):

An NMR method of analyzing an analyte comprises feeding an analyte sample fluid to an NMR flow cell. The NMR flow cell comprises an RF microcoil operably associated with an enlarged containment region. The mobile phase of the analyte sample flowing through the NMR flow cell has a solvent gradient greater than 10% per minute. The analyte sample fluid can be fed to the NMR flow cell from an analyte extraction chamber, e.g., operative to perform liquid chromatography, capillary electrophoresis, or the like, especially a capillary-based analyte extraction chamber integrated in an NMR probe with the NMR flow cell. A sample volume is held in the NMR flow cell for equilibration less than 1 hour, preferably less than 30 minutes prior to actuating NMR analysis of the observe volume in the microcoil.

Summary of Invention Paragraph (2):

[0001] This invention relates to methods and systems for NMR analysis of analytes in small volumes. More particularly, this invention relates to methods and systems employing NMR microcoils for analysis of analytes in, for example, nanoliter sample volumes. This application claims the benefit of U.S. Provisional Application No. 60/250,705, entitled STEEP SOLVENT GRADIENTS AND SAMPLE PRECONCENTRATION USING CAPILLARY LC-NMR, which was filed on Dec. 1, 2000.

Summary of Invention Paragraph (4):

[0002] Nuclear magnetic resonance spectroscopy, or NMR, is a powerful and commonly used method for analysis of the chemical structure of molecules. NMR provides spectral information as a function of the electronic environment of the molecule and is nondestructive to the sample. In addition, reaction rates, coupling constants, bond-lengths, and two- and three-dimensional structure can be obtained with this technique. NMR combined with liquid chromatography or capillary electrophoresis was demonstrated as early as 1978 using stopped flow (Watanabe et al. (1978) Proc. Jpn. Acad. 54:194), and in 1979 with continuous flow (Bayer et al. (1979) J. Chromatog. 186:497-507), though limitations due to solvent as well as inherent sensitivity curtailed the use of the method. That is, the inherent insensitivity of the NMR method limited its usefulness as a detection method for liquid phase analysis of very small samples, such as effluent from an analyte focusing or concentrating method (those two terms being used here interchangeably unless otherwise indicated), e.g., a liquid chromatography or capillary electrophoretic separation. Conventional NMR spectrometers typically use relatively large RF coils (mm to cm size) and samples in the μ l to ml volume range, and significant performance advantages are achieved using NMR microcoils when examining very small samples. NMR microcoils are known to those skilled in the art and are shown, for example, in U.S. Pat. No. 5,654,636 to Sweedler et al., and in U.S. Pat. No. 5,684,401 to Peck et al., and in U.S. Pat. No. 6,097,188 to Sweedler et al., all three of which patents are incorporated herein by reference in their entireties for all purposes. A solenoid microcoil detection cell formed from a fused silica capillary wrapped with copper wire has been used for static measurements of sucrose, arginine and other simple compounds. Wu et al. (1994a), J. Am. Chem. Soc. 116:7929-7930; Olson et al. (1995), Science 270:1967-1970, Peck (1995) J. Magn. Reson. 108(B) 114-124. Coil diameter has been further reduced by the use of conventional micro-electronic techniques in which planar gold or aluminum R.F. coils having a diameter ranging from 10-200 μ m were etched in silicon dioxide using standard photolithography. Peck 1994 IEEE Trans Biomed Eng 41(7) 706-709, Stocker 1997 IEEE Trans Biomed Eng 44(11) 1122-1127, Magin 1997 IEEE Spectrum 34 51-61, which are also incorporated herein by reference in its entirety for all purposes.

Summary of Invention Paragraph (5):

[0003] Miniature total analysis systems (.mu.-TAS) are discussed in Integrating Microfluidic Systems And NMR Spectroscopy--Preliminary Results, Trumbull et al, Solid-State Sensor and Actuator Workshop, pp. 101-05 (1998), Magin 1997 IEEE Spectrum 34 51-61, and Trumbull 2000 47(1) 1-6 incorporated herein by reference in its entirety for all purposes. The Trumbull et al. device integrated multiple chemical processing steps and the means of analyzing their results on the same miniaturized system. Specifically, Trumbull et al. coupled chip-based capillary electrophoresis (CE) with nuclear magnetic resonance spectroscopy (NMR) in a .mu.L-TAS system. Linewidths of 1.4 Hz have been demonstrated using single turn planar NMR coils integrated with microfluidic channels.

Summary of Invention Paragraph (6):

[0004] Capillary-based liquid chromatography and microcoil NMR have compatible flow rates and sample volume requirements. Thus, for example, the combination of the Waters CapLC.TM. available from Waters Corporation (Milford, Mass., USA) and the MRM CapNMR.TM. flow probe available from MRM Corporation (Savoy, Ill.), a division of Protasis Corporation (Marlborough, Mass., USA) provides excellent separation capability in addition to UV-VIS and NMR detection for mass-limited samples. The Waters CapLC.TM. has published flow rates from 0.02 .mu.L/minute to 40 .mu.L/minute. A typical CapLC on-column flow rate is 5 .mu..mu.L/min, the autosampler-injected analyte volume is 0.1 .mu.L or more, and accurate flow rates are achieved through capillary of typically 50 .mu.m inner diameter. The NMR flow cell has a typical total volume of 5 .mu.L with a microcoil observe volume of 1 .mu.L. A typical injected sample amount for CapLC-.mu.NMR analysis is a few .mu.g (nmol) or less.

Summary of Invention Paragraph (7):

[0005] Conventional scale LC-NMR systems (flow rates about 1 mL/min) typically employ solvent gradients of 2% per minute to 4% per minute. The probes have typical flow cell volumes of about 150 .mu.L and typical observe volumes of 30-60 .mu.L. In such systems, the application of a solvent gradient followed by stopped flow can initially result in severely distorted NMR line shapes due to the magnetic inhomogeneity caused by nonuniform solvent composition in the flow cell. After a reasonable equilibration time, if the solvent gradient is no longer too steep, line shape is seen to recover from the initial stopped flow conditions. However, steep solvent gradients typically result in magnetic distortions so severe that NMR is practically precluded due to the extremely long recovery time (>>1 hr) necessary to achieve high spectral resolution.

Summary of Invention Paragraph (8):

[0006] Capillary scale systems also are known. In such systems, a capillary-based analyte extraction chamber can be connected to an NMR flow cell, such as by being positioned as an operation site along a capillary channel extending to the NMR flow cell. Exemplary such integrated capillary-based analyte extraction chambers are shown in U.S. Pat. No. 6,194,900, the entire disclosure of which is incorporated herein by reference for all purposes.

Summary of Invention Paragraph (9):

[0007] There is a need in the art for improved NMR methods for analyzing analytes. There is a particular need for improved NMR methods of analyzing small samples, especially samples of less than 10 .mu.L or even less than 1.0 .mu.L.

Summary of Invention Paragraph (12):

[0009] Solvent gradients play an important role in NMR analysis, as indicated above. In accordance with the methods disclosed here, a flow of analyte sample fed to an NMR flow cell has a mobile phase with advantageously steep solvent gradient.

Summary of Invention Paragraph (13):

[0010] In accordance with a first aspect, a method of analyzing an analyte comprises feeding a fluid flow of analyte sample fluid, that is a fluid containing or suspected of possibly containing one or more analytes of interest in a multi-component mobile phase to a fluid channel of a nuclear magnetic resonance (NMR) probe. The analyte sample fluid is fed to an NMR flow cell in the fluid channel. The flow cell comprises an RF microcoil operably associated with an enlarged containment region. The mobile phase of the analyte sample flowing to the NMR flow cell has a solvent gradient greater than 10% per minute. In certain preferred embodiments, the mobile phase of the analyte sample fluid has a solvent gradient of 10%-30% per minute or more. Coupled with NMR detection, this variation

in (mobile phase) solvent composition is fed directly into the NMR flow cell. Exemplary mobile phases include aqueous/organic mobile phases, and any others suitable to the particular application, of which many are known to those skilled in the art. It will be recognized that the methods and systems disclosed here provide an advantageous technological advance. An operator of a liquid chromatography system or other analyte extraction means generally wants full access and control over the solvent composition of the analyte sample flow, as this chemistry directly influences the effectiveness of the separation. In many instances, the compositional mixture of two or more solvents will be intentionally varied to provide a desired effect. The necessary or useful rate of solvent compositional change is in some instances greater than 10% per minute, and the methods and systems disclosed here render such steep solvent gradients practical.

Summary of Invention Paragraph (14):

[0011] In accordance with another aspect, methods as disclosed immediately above further comprise holding a volume of the analyte sample fluid in the NMR flow cell for an equilibration time of less than 1 hour, preferably less than 30 minutes, e.g., 10 minutes, and then actuating NMR analysis of analyte in an observe volume of the NMR flow cell. Preferably the observe volume of the sample fluid in the NMR flow cell is less than 5 .mu.L, e.g., about 1 .mu.L. The sample volume may be held in the NMR flow cell, for example, by permanently or temporarily stopping the flow of analyte sample fluid in the NMR probe. In capillary-based embodiments of the systems and methods disclosed here, the analyte sample fluid has a fluid flow rate typically less than about 5 .mu.L/minute. In substrate-based, i.e., micro-scale, embodiments of the systems and methods disclosed here, the analyte sample fluid has a fluid flow rate typically less than 1 .mu.L/minute.

Summary of Invention Paragraph (15):

[0012] In accordance with another aspect, methods as disclosed immediately above further comprise processing sample fluid containing (here and in the appended claims meaning actually containing or suspected of possibly containing) an analyte in an analyte extraction device to produce analyte sample fluid that is then (optionally with one or more intervening processing steps) fed to the NMR flow cell comprising an RF microcoil. Exemplary analyte extraction devices include liquid chromatography (LC) devices, capillary electrophoretic and/or capillary electrochromatographic (CE/CEC) devices, electric field gradient focusing (EFGF, Koegler 1996 J. Chromatography 229 229-236, Koegler 1996 Biotech Prog 12(6) 822-836) and dynamic field gradient focusing (DFGF, Huang 1999 Anal. Chem. 71(8) 1628-1632) devices and the like. In certain preferred embodiments, sample fluid is processed in a capillary-based, i.e., a capillary scale, analyte extraction device, to produce analyte sample fluid that is then (optionally with one or more intervening processing steps) fed to a capillary NMR flow cell comprising an RF microcoil. Exemplary capillary scale analyte extraction devices include capillary LC and CE devices, e.g., the Waters CapLC.TM. column. A steep solvent gradient is then used to separate and remove analyte (e.g., an organic/water mobile phase with a 10-30% per minute gradient). The one or more analyte peaks are then individually stopped in the capillary NMR flow cell to acquire high resolution NMR data, e.g., a proton spectrum, after an equilibration time of typically less than 30 minutes. This capillary approach enables preconcentration of analytes on a separation column from relatively large volumes, having initial concentrations of typically less than 1 mM, and good separation efficiency coupled with good NMR spectral resolution. In particular, to make NMR practical as a means of detection for analytical separations it is highly advantageous that steep solvent gradients are used in conjunction with short equilibration times and yet good NMR spectral resolution is obtained.

Summary of Invention Paragraph (16):

[0013] In accordance with certain preferred embodiments, methods as disclosed above employ analyte sample volumes less than about 10 microliters. Exemplary such embodiments dispose analyte sample in an NMR flow cell wherein the inside dimension of the associated microcoil is less than about 1 mm. A static magnetic field is generated about the analyte sample using a magnet well known to those skilled in the art. Using NMR techniques well known to those skilled in the art, the free induction decay signal from the analyte is received by the NMR microcoil and preferably has a spectral linewidth of better than 0.1 parts per million (ppm), more preferably better than 0.01 ppm, e.g. 0.001 ppm.

Summary of Invention Paragraph (17):

[0014] In certain preferred embodiments, a miniaturized analysis system is employed for liquid phase sample analysis. Such methods and systems, referred to in some

instances here and in the appended claims as substrate-based methods and systems, employ: a microfabricated support body or manifold in the form of a cylinder, chip, laminated planar substrate or the like, having in or on the manifold one or more straight or branched microfabricated microchannels; optionally a housing, e.g., a cover plate, arranged over the manifold, optionally cooperating with the manifold to form sample processing channels or compartments; an inlet port for feeding fluid from an external source into the NMR probe; and an NMR flow cell comprising an NMR RF microcoil, in fluid communication with the inlet via one or more of the microfabricated microchannel. As used here, the terms "micro-scale" and "microfluidic" means the manifold operates effectively on micro-scale fluid samples, typically having volumes less than about 1 uL (i.e., 1 microliter), e.g., about about 0.1 microliter to 1.0 microliter, and fluid flow rates less than about 1 uL/min, for example 100 nanoliters/min. As used herein, the term "microscale" also refers to flow passages or channels and other structural elements of a substrate, e.g., a multi-layer laminated substrate. For example, 1 or more microchannels of the substrate preferably have a cross-sectional dimension (diameter, width or height) between 500 microns and 100 nanometers. Thus, at the small end of that range, the microchannel has cross-sectional area of about 0.01 square microns. Such microchannels within the laminated substrate, and chambers and other structures within the laminated substrate, when viewed in cross-section, may be triangular, ellipsoidal, square, rectangular, circular or any other shape, with at least one and preferably all of the cross-sectional dimensions transverse to the path of fluid flow is microscale. It should be recognized, that one or more layers of a laminated substrate may in certain embodiments have operative features, such as fluid channels, reaction chambers or zones, accumulation sites etc. that are larger than microscale. The substrates disclosed here provide effective microcoil NMR devices and systems with good speed of analysis, decreased sample and solvent consumption, increased detection efficiency, and in certain embodiments disposable fluid-handling devices.

Summary of Invention Paragraph (18):

[0015] The capillary-scale and micro-scale embodiments of the NMR methods and systems disclosed here provide significant commercial advantage over conventional (larger scale, e.g. larger than capillary) NMR systems including, for example: less sample fluid is required, which in certain applications can present significant cost reductions, both in reducing product usage (for example, if the test sample is a biological sample or is taken from a product stream) and in reducing the waste stream disposal volume. In addition, they can, in accordance with preferred embodiments, be produced employing MEMS and other known techniques suitable for cost effective manufacture of miniature high precision devices.

Summary of Invention Paragraph (19):

[0016] The methods disclosed here are carried out, in certain preferred embodiments, in a system embodied in an NMR probe comprising multiple processing sites (i.e., stages or devices operative to carry out fluid operations on a sample) integrated together in a common NMR manifold, such as capillary-based methods and systems or substrate-based methods and systems with fluid communication between the processing sites being provided by microchannels and the like defined by the manifold, i.e., formed in or on the manifold. The multiple processing sites in such systems and methods include at least an analyte extraction chamber and an NMR flow cell downstream of the analyte extraction chamber, i.e., positioned to receive analyte sample fluid from the analyte extraction chamber. For example, a biological sample, synthetic intermediary, metabolite, contaminant, natural product or the like is fed directly to the NMR probe. The sample is then prepared as required to produce analyte sample fluid, e.g., filtration, solid phase extraction, capillary electrophoresis, liquid chromatography, etc. The prepared analyte sample fluid is then passed to an NMR flow cell for NMR detection. The NMR detection chamber has an integrated NMR radio frequency microcoil embedded or otherwise integrated directly in the manifold. Following detection, the sample can be discarded or, optionally, stored in the NMR manifold indefinitely, either in the NMR flow cell or other chamber defined by the manifold. Optionally, the sample is transported via capillary or on-chip to a further analytical station or storage site. In certain preferred embodiments the total analysis requires less than about 1 .mu.L of sample.

Detail Description Paragraph (2):

[0017] The terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for

example, reference to "an analyte" includes mixtures of analytes, reference to "a detection means" includes two or more such detection means, reference to "a sample processing compartment" includes more than one such compartment, reference to "an NMR RF microcoil" includes two or more such microcoils, and the like.

Detail Description Paragraph (4):

[0019] The term "substrate," "manifold" and "support body" and the like are used interchangeably to refer to any material forms, incorporates or supports the NMR flow cell or other components, e.g. detection modules comprising a capillary onto which the microcoil is fabricated, or alternatively substrates that can be microfabricated, such as by being ablated, molded or embossed, to have desired configuration and features. A substrate can be a polymer, a ceramic, a glass, a metal, a composite of those and other materials, a laminate thereof, or the like. A "laminate" refers to a composite material formed from several different layers of the same or different materials.

Detail Description Paragraph (5):

[0020] The term "analyte sample fluid" refers to the fluid fed to the NMR flow cell. In preferred embodiments the analyte sample fluid is the direct or indirect product (i.e., with or without additional intervening processing) of a focusing or concentrating process performed on sample fluid containing (here meaning actually containing or suspected of possibly containing) an analyte. Such concentrating step, wherein an analyte of interest (e.g., reaction product, suspected pollutant or contaminant, chemical marker, etc.) has been concentrated from a larger quantity of a sample fluid (e.g., blood or other biological fluid, river water, product stream, reaction mixture, etc.) to produce analyte sample fluid, may be performed in an analyte extraction device and then (optionally with one or more intervening processing steps) fed to the NMR flow cell.

Detail Description Paragraph (7):

[0022] The term "analyte extraction chamber" means a column or other operative zone or site or the like for focusing or concentrating analyte from a sample fluid into an analyte sample fluid, typically involving a many-fold reduction in fluid volume. The analyte extraction chamber may be in a separate or stand-alone device, e.g., an LC column, with fluid communication to the NMR probe via any suitable conduit, e.g., a fluid delivery tube controlled by an autosampler. The analyte extraction chamber preferably is a capillary-based analyte extraction chamber and most preferably is integrated into the NMR probe, e.g., as an operation site along a fluid channel extending within the probe to the NMR flow cell in a substrate-based method or system. Preferably, the capillary-based analyte extraction chamber is operative to perform LC, CE/CEC, EFGF/DFGF, or the like. In the case of an LC column in the form of a capillary-based analyte extraction chamber operative to perform solid phase extraction (SPE) and integrated into the NMR probe in a substrate-based method or system, in some instances referred to here as an on-board LC chamber or on-board LC device, an analyte peak will be stepped off the column, i.e., released into the NMR probe fluid channel, when the relative proportion of analyte solvent (e.g., an organic solvent) in the analyte sample fluid reaches a sufficient concentration. As noted above, it is a significant and advantageous aspect of the methods disclosed here, that the relative proportion of analyte solvent is rapidly increased, resulting in a steep solvent gradient. Steep solvent gradients were avoided in the past due to the long equilibration time required for useful or precise NMR data. A measurable solvent gradient in the NMR observe volume during NMR data acquisition would impede useful or precise results; sample equilibration time is required sufficient to remove any significant solvent gradient. A steep solvent gradient, i.e., more than the conventional 2%-4% per minute, was seen to require unacceptably long equilibration time. In the methods disclosed here, steep solvent gradients are used and relatively short equilibration time is sufficient to remove any significant solvent gradient so as to permit acquisition of precise results. Thus, in the methods disclosed here, steep solvent gradients, e.g., greater than 10% per minute, e.g., 10%-30% per minute, and even greater than 30% or 50% per minute, enable fast step off of analyte from an analyte extraction chamber. Further, the analyte peak then is held in the NMR flow cell with its associated microcoil for only a short equilibration time to acquire high precision or high resolution NMR data. Without wishing to be bound by theory, it is presently understood that this phenomenon of fast equilibration of steep solvent gradient analyte sample fluid results from the high surface-to-volume ratio of the analyte sample fluid held in the NMR flow cell. Preferably, such phenomenon is achieved at the capillary scale and smaller, more preferably in structures where the inner diameter and/or channel width is less than about 1 mm... In any event, the NMR analysis methods disclosed enable rapid focusing

of analyte in an analyte extraction chamber by use of steep solvent gradients and yet enable short equilibration times in the NMR flow cell.

Detail Description Paragraph (8):

[0023] In certain preferred embodiments, equilibration of the steep solvent gradient in the NMR flow cell can be assisted to even more quickly achieve equilibration sufficient for accurate NMR data acquisition. That is, in addition to passive diffusion as disclosed above, auxiliary active means can advantageously be employed. Preferred auxiliary techniques for accelerating equilibration include elevating the temperature of the NMR flow cell, e.g., by applied heat, optical radiation, ultrasonic/mechanical vibration, etc., and ultrasonic mixing (as opposed to heating via sonication). Devices that can be employed on-board with the flow cell and/or remotely for generating and delivering heat, ultrasonic energy, etc. for such auxiliary equilibration are commercially available and will be apparent to those skilled in the art given the benefit of this disclosure.

Detail Description Paragraph (9):

[0024] An NMR flow cell, as that term is used here and in the appended claims, is an NMR site associated with an NMR microcoil, having an enlarged containment region, i.e., an enlarged void along a capillary-scale (also referred to as capillary-based) channel for holding a fluid sample in association with an RF microcoil. The inner diameter of the channel enlarges to form the enlarged void, preferably with conical tapering at each end of the void. The void may extend axially in the channel beyond the microcoil, such that the observe volume is smaller than the overall volume of the containment region. In accordance with certain preferred embodiments, the inner diameter of the channel on either side of the enlarged void is 5 μm to 500 μm , more preferably 25 μm to 50 μm , the conical taper of the channel at each end of the void preferably is at an angle to the longitudinal axis of about 5 to 75 degrees, e.g., about 30 degrees, and the inner diameter of the enlarged void between the conically tapered portions preferably is substantially uniform and from 100 μm to 1 mm, more preferably 250 μm to 800 μm . The microcoil is positioned to axially surround the void, typically being about 250 μm to 1 mm in the axial direction.

Detail Description Paragraph (10):

[0025] Preferred embodiments of the methods disclosed here provide NMR analysis suitable for elucidating the chemical structure of an analyte sample. Apparatus suitable for carrying out the methods disclosed here will be apparent to those skilled in the art given the benefit of this disclosure. Exemplary apparatus comprises an analyte sample holder having a containment region that holds a volume of less than about 10 microliters of the analyte sample; a microcoil, which encloses the containment region of the analyte sample holder and the analyte sample contained therein, the microcoil having an inside dimension of less than about 1 mm, and the microcoil operatively associated with the analyte sample contained in the containment region of the analyte sample holder such that the microcoil can transmit and/or receive energy from the analyte sample in the containment region of the analyte sample holder; and a magnet generating a static magnetic field about the analyte sample in the containment region of the analyte sample holder, wherein the microcoil and the magnet provide for the obtainment of an NMR spectrum of the analyte sample in the containment region of the analyte sample holder having a spectral line width of, e.g., less than about 0.1 parts per million.

Detail Description Paragraph (11):

[0026] The NMR flow cell preferably has a microcoil wrapped or wound around the above-mentioned enlarged zone that serves as the sample holder. As noted above, the observe volume, i.e., the volume of sample actually coupled to the microcoil and subjected to NMR analysis, typically is substantially less than the total volume of the enlarged zone. Preferably the NMR flow cell has a total volume less than about 10 microliters, more preferably about 5 microliters, with a microcoil observe volume, e.g., of about 1 microliter. The sample holder may be a capillary, which can be formed from many different materials. In selecting a sample holder, the material from which the sample holder is constructed should not detrimentally interact with the analyte or interfere with the operation of the microcoil or the magnet. A preferred sample holder is a capillary formed from fused silica (or glass) with an inside dimension in the containment region of between 50 and 1000 μm . Preferably, the shape of the capillary is cylindrical; however, other geometric shapes may also be used. In a preferred embodiment, the microcoil is wrapped about the outside surface of the capillary, and thus, the capillary serves as both the sample holder and the coil form. In a substrate-based method or system the sample holder can be formed in or on a substrate which has one or more channels or grooves with an

enlarged area to serve as the analyte sample holder, i.e., as the flow cell. The channels or grooves may be introduced in the substrate by etching or the like.

Detail Description Paragraph (12):

[0027] Systems suitable for carrying out the methods disclosed here typically will have an electrical circuit in operable association with the microcoil to transmit to and receive energy from the analyte sample in the flow cell. A detection circuit typically includes impedance matching network components that are made out of materials that are designed to minimize susceptibility induced line broadening in the NMR spectrum by minimizing the static magnetic field distortions. In addition, the same electrical circuit can serve to transmit detected energy from the sample and introduce energy or signals to a processor. The processor which analyzes the NMR signals may be on-board or remote and optionally drives a graphical display device to show detected energy as a time or frequency domain NMR spectrum. The types of circuits and processors that accomplish this are well known to those skilled in the art, and many variations can be used in connection with the present invention depending on the desired applications and types of NMR spectra desired. Such processors may include computers and any associated software, which are also known in the art.

Detail Description Paragraph (13):

[0028] Typically, the microcoil is physically separated from the electrical circuit, although the two are electrically connected. Separation of the microcoil and the analyte from the components of the electrical circuit tends to minimize the susceptibility induced distortions of the static magnetic field in the flow cell. It is to be understood that other arrangements for matching the impedance and reducing local distortions can be used and that electrical performance and signal-to-noise (SNR) advantages may be obtained using these other configurations known to those skilled in the art. For example, the impedance matching elements can be positioned immediately adjacent to the microcoil.

Detail Description Paragraph (14):

[0029] Suitable magnets for use in methods disclosed here are commercially available and well known to those skilled in the art. The magnets generally are high strength superconducting magnets, although other magnets (e.g., permanent or resistive) may be used. The magnet generates a static, homogeneous magnetic field about the microcoil and the analyte sample in the flow cell. Additional sets of (shim) coils are incorporated to provide correction of magnetic field inhomogeneities, typically improving magnetic field homogeneity by 100-1000 fold over that of a "virgin magnet." Such systems are well known to those skilled in the art.

Detail Description Paragraph (15):

[0030] Substrate-based method and systems disclosed here can be carried out on integrated manifolds, optionally incorporating operative devices fluidically coupled to the NMR flow cell of the manifold. Suitable production techniques for making such miniature, integrated devices are well known to those skilled in the art and include, for example, silicon micromachining techniques and etching techniques to create miniaturized columns in a wide variety of polymeric, ceramic, glass, metal and composite substrates having suitable characteristics, e.g., resistance to chemical attack, dimensional stability, etc. Other suitable techniques include, e.g., ablating, molding or embossing component microstructures in a substrate using processes and materials well known in the art.

Detail Description Paragraph (16):

[0031] While certain preferred embodiments are described above of the steep solvent NMR system and analysis methods disclosed here, such description is intended to illustrate and not limit the scope of the invention. Other aspects and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains, and the following claims are intended to cover all those aspects and modifications within their terms.

CLAIMS:

1. A method of analyzing an analyte, comprising feeding a fluid flow of analyte sample fluid comprising analyte in a mobile phase to a fluid channel of a nuclear magnetic resonance (NMR) probe comprising an NMR flow cell in the fluid channel, the flow cell comprising an RF microcoil operably associated with an enlarged containment region, wherein the mobile phase of the analyte sample flowing through the NMR flow cell has a solvent gradient greater than 10% per minute.

2. The method of analysis of an analyte in accordance with claim 1 wherein the NMR flow cell is in a capillary-scale fluid channel.
3. The method of analysis of an analyte in accordance with claim 1 wherein the NMR flow cell is in a micro-scale fluid channel.
6. The method of analysis of an analyte in accordance with claim 1 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber.
7. The method of analysis of an analyte in accordance with claim 6 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform liquid chromatography.
8. The method of analysis of an analyte in accordance with claim 6 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform electric field gradient focusing.
9. The method of analysis of an analyte in accordance with claim 6 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform dynamic field gradient focusing.
10. The method of analysis of an analyte in accordance with claim 6 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform electrochromatography.
11. The method of analysis of an analyte in accordance with claim 6 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from a capillary-based analyte extraction chamber.
12. The method of analysis of an analyte in accordance with claim 11 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from a capillary-based liquid chromatography chamber.
13. The method of analysis of an analyte in accordance with claim 12 wherein the capillary-based liquid chromatography chamber is integrated in the NMR probe in fluid communication with the NMR flow cell.
14. The method of analysis of an analyte in accordance with claim 11 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform capillary electrophoresis.
15. The method of analysis of an analyte in accordance with claim 11 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform capillary electrochromatography.
16. The method of analysis of an analyte in accordance with claim 11 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform capillary-scale electric field gradient focusing.
17. The method of analysis of an analyte in accordance with claim 11 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform capillary-scale dynamic field gradient focusing.
19. The method of analysis of an analyte in accordance with claim 11 wherein the observe volume of the sample fluid in the NMR flow cell is less than 5 .mu.L.
20. The method of analysis of an analyte in accordance with claim 6 wherein the NMR flow cell is fed from a micro-scale analyte extraction chamber.
21. The method of analysis of an analyte in accordance with claim 20 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from a micro-scale liquid chromatography chamber.
22. The method of analysis of an analyte in accordance with claim 20 wherein the micro-scale liquid chromatography chamber is integrated in the NMR probe in fluid

communication with the NMR flow cell.

23. The method of analysis of an analyte in accordance with claim 20 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform micro-scale electrophoresis.

24. The method of analysis of an analyte in accordance with claim 20 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform micro-scale electrochromatography.

25. The method of analysis of an analyte in accordance with claim 20 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform micro-scale dynamic field gradient focusing

26. The method of analysis of an analyte in accordance with claim 20 wherein the fluid flow of analyte sample fluid to the NMR flow cell is fed from an analyte extraction chamber operative to perform micro-scale electric field gradient focusing.

27. The method of analysis of an analyte in accordance with claim 20 wherein the observe volume of the sample fluid in the NMR flow cell is less than 1 .mu.L.

25. The method of analysis of an analyte in accordance with claim 1 further comprising stopping flow of the analyte sample fluid with a fluid sample volume of the analyte sample fluid in the NMR flow cell for an equilibration time of less than 30 minutes and then actuating NMR analysis of analyte in an observe volume of the sample fluid in the NMR flow cell.

26. The method of analysis of an analyte in accordance with claim 25 wherein the equilibration time is less than 20 minutes before actuating NMR analysis of analyte in the observe volume of the sample fluid in the NMR flow cell.

27. The method of analysis of an analyte in accordance with claim 25 wherein the equilibration time is less than 10 minutes before actuating NMR analysis of analyte in the observe volume of the sample fluid in the NMR flow cell.

28. A method of analyzing an analyte, comprising feeding analyte sample fluid to an NMR flow cell comprising an RF microcoil operably associated with an enlarged containment region, wherein the analyte sample has a solvent gradient greater than 10% per minute.

L7: Entry 15 of 21

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TITLE: Integrated miniaturized device for processing and NMR detection of liquid phase samples

Abstract Text (1):

A miniaturized total analysis system with an in-line NMR detection compartment and an NMR rf microcoil detector is described for use in liquid phase analysis. The device is formed by microfabrication of microstructures in novel support substrates. The NMR detector coil may be fabricated directly in the support body at the point of detection or, alternatively, may be formed as part of a modular structure that is insertable into the device at the point of detection. In addition, an integrated device for sample preparation and NMR detection is provided comprising the miniaturized total analysis system and a miniature magnet configured to accept the miniaturized total analysis system, wherein the device is capable of generating an NMR spectrum. The invention herein is used for the analysis of small and/or macromolecular and/or other solutes in the liquid phase.

Brief Summary Text (2):

The present invention relates generally to miniaturized liquid phase sample processing and analysis. More particularly, the invention relates to a miniaturized planar sample preparation and analysis device with an integrated on-chip miniature nuclear magnetic resonance ("NMR") radiofrequency coil. Both the sample preparation and analysis device and the NMR radiofrequency coil are manufactured using a variety of means suitable for microfabrication of substrate materials such as, but not limited to ablation, molding and embossing. The device is intended to be used with high-field, low-cost miniature magnets with the intention of achieving high throughput, fast time-to-result analysis of biological liquids in a truly integrated fashion.

Brief Summary Text (4):

Real time identification of analytes in a complex biological fluid is difficult, and requires careful thought as to (a) the preparation of the sample, (b) whether a separation step is required to simplify the signal, (c) whether a detection method can be employed which has no effect on the sample itself, (e.g. non-destructive). An ideal device would allow rapid detection of a wide range of simple or complex molecules in the liquid phase, at biological concentrations, and yield information about chemical structure and composition. A desirable feature of the detection method would be to enable the separation criteria to be relaxed such that sample preparation and detection could occur in series, without the need for complex separation technology. An on-line detector is particularly advantageous when sample size is limited, and additional analysis of the sample is required. Moreover, mass spectrometry ("MS") and NMR are detection methods well suited to yielding high quality chemical information for multi-component samples, requiring no a priori knowledge of the constituents.

Brief Summary Text (7):

One of the most powerful analytical methods for molecular structure information is NMR. NMR provides spectral information as a function of the electronic environment of the molecule and is nondestructive to the sample. In addition, reaction rates, coupling constants, bond-lengths, and two- and three-dimensional structure can be obtained with this technique. The strength of both NMR and MS is the ability to derive fundamental chemical structure information, which is high resolution in terms of either chemical shift or mass, yielding the possibility of simultaneous analysis of multiple species. The inherent insensitivity of the NMR method however, has limited its usefulness as a detection method for liquid phase analysis of very small

samples, such as effluent from a liquid chromatography or capillary electrophoretic separation.

Brief Summary Text (8):

NMR combined with liquid chromatography or capillary electrophoresis was demonstrated as early as 1978 using stopped flow (Watanabe et al. (1978) Proc. Jpn. Acad. 54:194), and in 1979 with continuous flow (Bayer et al. (1979) J. Chromatog. 186:497-507), though limitations due to solvent as well as inherent sensitivity curtailed the use of the method. See Dorn et al. (1984) Anal. Chem. 56:747-758 for a review.

Brief Summary Text (9):

Recent experiments using NMR as a detector for nanoliter sample volumes, suggests that NMR could provide a greater detection sensitivity than in previous investigations. Wu et al. (1994a) J. Am. Chem. Soc. 116:7929-7930; Olson et al. (1995) Science 270:1967-1970; Wu et al. (1994b) Anal. Chem. 66:3849-3857; and Wu et al. (1995) Anal. Chem. 67:3101-3107. Unfortunately, though observations were made on nanoliter volumes, the findings translate into millimolar levels of detection sensitivity.

Brief Summary Text (10):

A number of areas can be targeted to increase the sensitivity of NMR detection for liquid phase analysis. Resistive losses, operating temperature, sample ionic strength, filling factor, and coil geometry affect the sensitivity of the coil. Cooling the radiofrequency coil and using superconducting coil material have resulted in some gain in signal-to-noise through reduction in coil resistance and thermal properties. However, it is difficult to achieve the theoretical maximum, since detecting signal from a room temperature liquid sample using a cryogenically cooled radiofrequency probe has proven difficult.

Brief Summary Text (11):

The NMR signal-to-noise is directly proportional to the sample volume ($V_{\text{sub.s}}$) interrogated by the detection coil (filling factor), the magnetization per unit volume ($M_{\text{sub.o}}$), and the strength of the radiofrequency ("RF") field ($B_{\text{sub.1}}$) per unit current, and inversely proportional to the square root of the coil resistance (R):

Brief Summary Text (12):

Signal-to-noise can be maximized by decreasing the coil radius, and matching the coil inner diameter as close to the size of the sample as possible. Inadequate filling factor will generally be an issue when standard radiofrequency NMR coils are used to detect signal from very small sample volumes, e.g., from a microcolumn or other miniaturized sample preparation technology. Reduction in the size of NMR radiofrequency coils to the diameter of the fused glass capillary used for these types of separations, has allowed detection of signal from nanoliter volumes from on-line capillary electrophoretic separations Wu et al. (1994a), supra; Olson et al. (1995), supra; Wu et al. (1994b), supra; Wu et al. (1995), supra.

Brief Summary Text (13):

A solenoid microcoil detection cell formed from a fused silica capillary wrapped with copper wire has been used for static measurements of sucrose, arginine and other simple compounds. Wu et al. (1994a), supra; Olson et al. (1995), supra. Coil diameter has been further reduced by the use of conventional micro-electronic techniques in which planar gold or aluminum R.F. coils having a diameter ranging from 10-200 μm were etched in silicon dioxide using standard photolithography. Peck (1995) J. Magn. Reson. 108(B) 114-124. The signal-to-noise ratio (SNR) of these planar micro-coils for analyzing solid samples, e.g., silicon rubber was increased by a factor of 10 over other coils. For significant advancement in hyphenating NMR with LC or CE methods, an approach allowing micromolar or even nanomolar limits of detection is required however.

Brief Summary Text (14):

Factors affecting the limit of detection can also be attributed to bulk susceptibility shifts, which become dominant when the sample volume is of the order of the size of the sample chamber and coil. This is in addition to the coil geometry, resistive losses, sample ionic strength, filling factor and operating temperature considerations previously mentioned. We have constructed susceptibility-matched microcoils using 50 μm copper wire with an inner diameter of 70 μm , and obtained signal in 64 seconds from a 12.5 mM solution of arginine

at 400 MHZ, with a signal-to-noise of 6:1. Or, in other words, normalizing these results to 300 MHZ for direct comparison with U.S. Pat. No. 5,654,636, issued Aug. 5, 1997, to Sweedler et al. this yields a signal-to-noise of 3:1 versus 1:1 obtained in Sweedler et al.

Brief Summary Text (16):

These calculations indicate that an integrated system could be constructed with the ability to detect micromolar quantities of analyte contained in nanoliter samples using in-line NMR detection following sample preparation and separation.

Brief Summary Text (17):

While silicon micro-machining has been useful in the fabrication of miniaturized liquid phase analysis systems, improvements have been made to overcome the inherent shortcomings of this technique. For example, U.S. Pat. No. 5,500,071 to Kaltenbach et al., U.S. Pat. No. 5,571,410 to Swedberg et al and U.S. patent application Ser. No. 08/656,281 to Kaltenbach et al., disclose the use of laser ablation to form microstructures in novel polymer substrates. This permits an enhanced symmetry and alignment of structures formed by component parts, enhanced separation capabilities, avoidance of problems with SiO₂ chemistry, low-cost manufacturing, the formation of microstructures of any size and geometry, and the incorporation of a detection means for on-column analysis. The advantage of combining miniaturized planar liquid phase analysis systems with on-column NMR detection is clearly advantageous in terms of lower overhead for instrument maintenance, increased speed of analysis, decreased sample and solvent consumption, full automation capabilities, increased detection efficiency, and increased quality of information.

Brief Summary Text (18):

A method and an apparatus for NMR spectroscopy of samples from online separation methods has been described. Sweedler et al., supra. While the problems of susceptibility and signal-to-noise from samples of an online separation apparatus are addressed therein, the method and apparatus described is limited to analysis of simple aqueous solutions. The apparatus includes a capillary channel etched or grooved in a substrate such as glass or polycarbonate and a planar lithographic microcoil. The use of micron-feature devices with integrated sample preparation and detection is not described. Integration of the NMR coil with the separation device eliminates dead volume which increases the dispersion and drastically degrades resolution between the point of chemical separation and detection. The method and apparatus presented in Sweedler et al. uses a conventional NMR spectrometer, such that sample preparation occurs outside of the separation/detection system, and hence a truly integrated solution for sample preparation and detection is lacking.

Brief Summary Text (20):

There is yet a need in the art for a miniaturized device that avoids the problems of chemical and pH instability that are typical with SiO₂ substrates and has miniaturized liquid sample handling capabilities and on-board sample preparation and NMR detection means.

Brief Summary Text (22):

Accordingly, it is a primary object of the invention to provide a novel miniaturized separation system with an integrated NMR detection chamber and an NMR rf microcoil detector for on-line NMR analysis of samples separated.

Brief Summary Text (23):

It is another object of the invention to provide a miniaturized total analysis system for liquid phase analysis comprising a miniaturized sample processing device having online separation and detection by NMR for liquid phase analysis.

Brief Summary Text (24):

It is yet another object of the invention to provide an integrated device for sample preparation and NMR detection, to be used with high field, low cost miniaturized magnets.

Brief Summary Text (26):

The present invention is directed to a novel miniaturized total analysis system for liquid phase sample preparation and detection. The system comprises an NMR detection compartment and an NMR rf microcoil detector.

Brief Summary Text (27):

The inventors are not aware of any integrated miniaturized system comprising sample

processing including sample pretreatment, separation and NMR detection integrated with an NMR rf microcoil for liquid phase analysis. Accordingly, the invention, as now provided, represents a novel and important advance in liquid phase analysis using miniaturized devices. It has been found by the inventors that the integration of an NMR rf microcoil detector with the processing device as now provided eliminates dead volume between the point of chemical preparation and processing and point of detection, and thereby obviates a common source of artifact and signal acquisition delay encountered with stand-alone processing devices interfaced with an NMR instrument. In addition, on-line analysis with the NMR rf microcoil detector enables a faster time-to-result and analysis with increased sensitivity of detection.

Brief Summary Text (32):

downstream from the sample processing compartment and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil.

Brief Summary Text (38):

downstream from the elongate bore and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil. The NMR detection compartment and the NMR rf microcoil may be fabricated directly in the support body at the point of detection. Alternatively, the NMR detection compartment and the NMR rf microcoil are formed in an insertable modular structure.

Brief Summary Text (40):

In still a further embodiment, an integrated system for sample preparation and NMR detection is provided comprising the aforementioned miniaturized total analysis system.

Brief Summary Text (41):

The system comprises microchannels and chambers for sample preparation, separation and detection. For example, a biological sample such as blood, urine, milk, cell or tissue extract, fermentation product or the like is added directly to the planar device. The sample is then prepared as required for the particular separation process to be performed, i.e., filtration, solid phase extraction, capillary electrophoresis or liquid chromatography. The prepared sample is then shunted to a separation chamber, and immediately following separation, detected in an NMR detection chamber. The NMR detection chamber has an integrated NMR radiofrequency coil embedded directly in the support media. Following detection, the sample can be discarded or, optionally, transported on chip to a further analytical station. The total analysis would require less than 1 .mu.L of sample.

Brief Summary Text (42):

The integrated system therefore, provides on-device sample preparation, separation and detection, as well as a transport medium for further analysis if required, an important feature when sample volumes of less than 1 .mu.L are to be handled. The miniaturized separation device can be formed from a polymer support body having essentially planar halves with microchannels and apertures fabricated therein. When aligned, the two halves define a separation compartment having inlet and outlet ports, an NMR detection chamber and an NMR radiofrequency coil.

Brief Summary Text (43):

In yet another embodiment, an integrated device for sample preparation and NMR detection is provided. The device comprises the aforementioned miniaturized total analysis system and a magnet configured to accept the a miniaturized total analysis system, wherein the device is capable of generating an NMR spectrum. The integrated device with NMR micro-coil is positioned within the center of the homogeneous field of the magnet, which is comprised of: (1) a miniature, e.g., table-top, magnet, such as is currently manufactured by American Magnetics Corp, of field strength of at least 300 MHZ to 750 MHZ; (2) associated electronics and data acquisition and storage capabilities for acquisition of, display and storage of multinuclear NMR spectra acquired from the rf microcoil; and (3) appropriate shielding of the NMR magnet so that the ensemble, i.e., the liquid phase analysis device, the NMR micromagnet and associated peripherals, can be situated easily on a table top or counter.

Brief Summary Text (45):

In still another embodiment, the micro-magnet, and miniaturized device for processing and NMR detection can be coupled with commercially available sample preparation or separation devices in which the micro-magnet coupled with the planar

NMR detection device functions primarily as a liquid handling device for very small sample volumes. The sample treatment chamber in the miniaturized sample-processing device can be used to mix in NMR active labels for selective detection in the NMR detection chamber. Following NMR detection the micro-analysis system can then be used to transport the sample following NMR detection, to another detection or analysis method, such as MS.

Brief Summary Text (50):

Laser ablation or other microfabrication techniques used in a planar substrate allows for formation of microstructures of almost any geometry or shape. This feature not only enables the formation of complex device configurations, but further allows for integration of sample injection, sample preparation, pre- or post-separation chemical modification and a variety of detection means, in particular, an NMR detection means, in a miniaturized total analysis system of greatly reduced overall dimensions.

Drawing Description Text (30):

FIG. 24 is a pictorial representation of a miniaturized total analysis system with an NMR detection compartment and an NMR rf microcoil. FIG. 24A illustrates a miniaturized total analysis system in which the NMR detection compartment and the NMR rf microcoil are fabricated in the support body. FIG. 24B illustrates a miniaturized total analysis system in which the NMR detection compartment and the NMR rf microcoil are components of a modular structure removably insertable into the support body. In addition, FIG. 24A schematically illustrates a separation device with on-board transmit-receive circuitry, while FIG. 24B illustrates the device with on-board receive-only circuitry.

Drawing Description Text (31):

FIG. 25 schematically illustrates a separation device of this invention interfaced with a micromagnet NMR spectrometer.

Detailed Description Text (2):

Before the invention is described in detail, it is to be understood that this invention is not limited to the particular component parts of the devices described or process steps of the methods described as such devices and methods may vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an analyte" includes mixtures of analytes, reference to "a detection means" includes two or more such detection means, reference to "a sample processing compartment" includes more than one such compartment, reference to "an NMR rf microcoil" includes two or more such microcoils, and the like.

Detailed Description Text (11):

As used herein, the term "NMR rf detector" or "NMR detection means" refers to any means, structure or configuration which allows one to interrogate a sample within an on-device NMR microcoil using an external magnet. Thus, an NMR detection means an NMR microcoil that communicates with the sample processing compartment and allows an external magnet to be interfaced with the sample processing compartment to detect an analyte passing through the compartment.

Detailed Description Text (33):

The term "NMR rf coil" is used herein to refer to a radiofrequency resonator producing a magnetic field (B._{sub.1}) orthogonal to the main magnetic field (B._{sub.0}).

Detailed Description Text (34):

The term "NMR rf microcoil" is used herein to refer to an NMR rf coil having a cross-sectional area less than 1 mm.²

Detailed Description Text (35):

The term "susceptibility" is used herein to refer to the ratio of magnetization of a material to the magnetic field strength. Magnetic susceptibility differences between the sample to be analyzed and other diamagnetic materials interposed between the sample and magnetic field can introduce magnetic field inhomogeneities, and result in broadened spectral lines. Susceptibility differences can be reduced by matching the magnetic susceptibility of the medium surrounding the sample to that of the coil

material, e.g., by surrounding the coil and coil-NMR detection chamber interface with susceptibility matching fluids such as Fluorinert.RTM., a perfluorinated organic liquid (3M, St. Paul, Minn.).

Detailed Description Text (36):

The term "NMR detection chamber" is used herein to refer to a sample detection chamber where resonant nuclei are interrogated by the radiofrequency coil.

Detailed Description Text (40):

Accordingly, the invention concerns formation of integrated miniaturized sample processing devices including NMR detection means using microfabrication techniques in a suitable substrate. It is also contemplated to form sample processing devices and NMR detection means according to the invention using injection molding techniques wherein the original microstructure has been formed by an excimer laser ablation process, or where the original microstructure has been formed using a LIGA process.

Detailed Description Text (46):

In a preferred embodiment of the invention, channels of a semi-circular cross section are laser ablated by controlling exposure intensity or by making multiple exposures with the beam being reoriented between each exposure. Accordingly, when a corresponding semi-circular channel is aligned with a channel thus formed, a sample processing chamber of highly symmetrical circular cross-section is defined which may be desirable for enhanced fluid flow through the sample processing device.

Detailed Description Text (56):

It is noted that the ablated by-pass channel 24 and apertures 26 and 28 further enable a wide variety of sample introduction techniques to be practiced according to the invention. Particularly, having a by-pass channel which is not connected to the sample processing compartment allows a user to flush a sample through the by-pass channel without experiencing sample carry-over or column contamination. As will be appreciated by one of ordinary skill in the art after reading this specification, one such sample introduction technique may be effected by butt-coupling an associated rotor to a stator (not shown) on the external surface of a miniaturized column where the rotor selectively interfaces external tubing and fluid sources with inlet port 18 and apertures 26 and 28, allowing a sample to be flushed from the by-pass channel 24 into external tubing from which the sample may then be introduced into the column via inlet port 18 for liquid phase analysis thereof. In this regard, a miniaturized column device formed in a polyimide substrate enables a ceramic rotor, pressed to the device using tensioned force (to form a liquid-tight seal), to still rotate between selected aperture positions on the device due to the friction characteristics of the two materials. Other suitable rotors can be formed in rigid materials such as, but not limited to, glass and non-conductive substrates.

Detailed Description Text (91):

Referring now to FIGS. 10 and 11, the miniaturized column device 102 further comprises detection means laser ablated in the support body 104. More particularly, a first aperture 128 is ablated in said first component half 106 and communicates with the first microchannel 114 at a point near the second end 126 thereof. A second aperture 130 is likewise formed in said second component half 108 to communicate with the second microchannel 116. Accordingly, a wide variety of associated detection means, e.g., NMR detection means, may then be interfaced to the sample processing compartment 118 to detect separated analytes of interest passing therethrough, such as by connection of electrodes to the miniaturized column via the first and second apertures 128 and 130.

Detailed Description Text (103):

The manifold 194 can be coupled to the cover plate 196 to form a liquid-tight interface using pressure sealing techniques known in the art. The manifold and cover plate can be mechanically urged together using clips, tension springs or any suitable clamping means known in the art. The manifold 194 generally includes a plurality of ports that are configured to correspond with the pattern of apertures and inlets present in the cover plate 196. Referring particularly to FIG. 16, a first conduit 204 can be used to interface an associated containment means (not shown) housing a sample to be separated, or a suitable buffer, with the separation channel 206. The conduit 204 is interposed within a port 208 in the manifold 194, and arranged to be in fluid communication with the upstream terminus 210 of the separation channel 206 via the inlet port 200. In this manner, fluids from the associated containment means can be readily delivered to the separation compartment

using known injection methods.

Detailed Description Text (107):

The liquid phase separation apparatus 190 may further include detection means, disposed in the cover plate 196 and/or the substrate portion 214. The detection means can comprise one or more apertures or features that have been laser-ablated in the cover plate or substrate portion, e.g., the NMR detection chamber, and communicate with the separation compartment at a position adjacent to, or substantially nearby, the downstream terminus 232 of the separation channel 206 to enable the detection of separated analytes. Referring to FIGS. 10 and 11, one particular apparatus includes an aperture 234 that is ablated in the substrate portion 214 and communicates with the separation channel 206 near the downstream terminus 232 thereof. A second aperture 236 is ablated in the cover plate 196, and is arranged to be in coaxial alignment with the aperture 234 when the cover plate is aligned over the substrate as has been described above. The coaxial apertures allow electrodes to be connected to the miniaturized column device 198 via the subject corresponding apertures to detect separated analytes of interest passing through the separation compartment by electrochemical detection techniques. In one particular apparatus, the coaxially aligned apertures form an optical detection path, enabling the optical detection of separated analytes passing through the separation compartment. As will be appreciated by those skilled in the art, an NMR detection device and/or a wide variety of associated optical detection devices can be interfaced with the separation compartment via the coaxial apertures, enabling the practice of spectrophotometric techniques such as UV/Vis, fluorescence, refractive index (RI), Raman and the like to detect separated analytes in the liquid sample.

Detailed Description Text (109):

The column device 304 also includes a makeup flow channel 320 laser-ablated in the planar surface 312. A makeup flow compartment is formed by the combination of the cover plate 316 and the makeup flow microchannel 320. The makeup flow channel has an upstream terminus, 322, which is in fluid communication with a makeup inlet port 324, comprising an aperture laser-ablated in the cover plate 316 and arranged to communicate with the terminus when the cover plate is positioned over the column substrate.

Detailed Description Text (111):

Referring particularly to FIG. 14, the manifold 306 includes a first port 326, a second port 328, a third port 330 and a fourth port 332, each port being configured to accept an associated conduit means 334, 336, 338, and 340, respectively. The conduit means are in fluid communication with associated fluid containment means (not shown), such that a fluid sample, reagent or buffer can be communicated to the various ports in the manifold 306 for delivery into the column device 304. Referring now to FIGS. 14 and 15A, when the manifold 306 is in a first position, the first manifold port 326 is in fluid communication with the upstream terminus 308 of the separation channel 310. In this position, a suitable liquid medium, such as an equilibrating buffer or a flush solution, can be delivered into the separation compartment (at the upstream terminus 308) from an associated containment means via the conduit means 334. Further, when the manifold is in the first position, the third manifold port 330 is in fluid communication with the upstream terminus of the makeup flow channel 320. Thus, a suitable liquid medium can be delivered into the makeup flow compartment (at the upstream terminus 322) from the same, or a different associated containment means, via the conduit means 338.

Detailed Description Text (112):

Referring now to FIGS. 14 and 15B, when the manifold 306 has been rotated counter-clockwise about the stator to a second position relative the column device 304, the fourth manifold port 332 is brought into fluid communication with the upstream terminus 308 of the separation channel 310. Accordingly, a volume or aliquot of liquid sample can be delivered into the separation compartment (at the upstream terminus 308) from an associated sample containment means via the conduit means 340. When the manifold is arranged in the second position, the first and third manifold ports 326 and 330 are moved out of fluid communication with the separation compartment and the makeup fluid compartment such that liquid medium is no longer delivered into those compartments via conduit means 334 and 338. Further, in the second position, the second manifold port 328 is aligned to be in fluid communication with the upstream terminus 322 of the makeup fluid channel 320, and a liquid reagent, or a heated makeup fluid can be delivered into the makeup flow compartment (at the upstream terminus 322) from an associated sample containment means via the conduit means 336.

Detailed Description Text (116) :

A liquid phase separation apparatus may be provided having a movable manifold, wherein the manifold cooperates with an on-device volumetric sample compartment (e.g., a covered bypass channel in fluid communication with inlet and outlet means as described above), to enable the delivery of a sample plug of known volume from the sample compartment to the upstream terminus of a separation compartment. The manifold is detachably coupled to a miniaturized column device, and arranged in a first position such that external conduits disposed within two ports of the manifold enable dynamic fluid communication between the sample compartment (via the inlet and outlet means) and an associated sample containment means. A sample plug, having a volume corresponding to the dimensions of the volumetric sample compartment, is formed by the dynamic flow of sample through the compartment. By moving the manifold to a second position, different ports in the manifold are brought into fluid communication with the volumetric sample compartment inlet and outlet, whereby those ports allow the flow of an externally housed liquid medium through the sample compartment and into the separation compartment via associated conduits and/or lateral ports in the manifold. In this manner, the sample plug disposed within the volumetric sample compartment can be readily delivered to the separation compartment using known liquid injection techniques.

Detailed Description Text (118) :

A separation compartment is formed by arranging the cover plate 358 over the planar surface of the substrate portion 356. The cover plate includes a plurality of apertures that are arranged to provide fluid communication with the separation compartment and the microchannels 364, 366 and 368 when the cover plate is in place above the substrate. Specifically, laser-ablated apertures 382 and 390, are respectively in fluid communication with the first and second terminus, 370 and 372, of the microchannel 364 to provide a first flow path. Laser-ablated apertures 384 and 392 are respectively in fluid communication with the first and second terminus, 378 and 380, of the microchannel 368 to provide a second flow path. A third flow path is provided by apertures 388 and 394, that are respectively in fluid communication with the first and second terminus, 374 and 376, of the microchannel 366. An aperture, 386, is in fluid communication with the upstream terminus 362 of the separation channel 360.

Detailed Description Text (123) :

The present invention combines miniaturized device technology described above combined with NMR detection in a single fabricated device wherein, using techniques described herein, a wide variety of microstructures may be formed, as will be appreciated by those working in the field of liquid phase analysis devices. The miniaturized device includes an NMR rf microcoil in series with the separation compartment at the point of detection. The microcoil can be fabricated directly into the separation device, as illustrated in FIG. 24A and as disclosed in the Example which follows. Alternatively, as shown in FIG. 24B, a microcoil-containing module can be manufactured separate from the separation device and inserted into the device prior to use to give a liquid-tight seal with zero dead volume.

Detailed Description Text (124) :

The NMR coil is formed integral with or as an attachment to the support body in any of a variety of ways that provide a coil geometry well known in the art. Any coil geometry can be used in the device that allows the radiofrequency field generated by the coil to be perpendicular to the main magnetic field.

Detailed Description Text (130) :

FIGS. 24A and 24B illustrate a miniaturized device as illustrated in FIG. 10 further comprising an integrated microcoil and a miniaturized column device with a microcoil-containing module, respectively. The device, generally indicated at 500A and 500B, comprises a support body 502A and 502B having first and second component halves indicated at 504A, 504B and 506A, 506B, respectively. The first and second component halves 504A, 504B and 506A, 506B, each having substantially planar interior surfaces (not illustrated) into which microchannel has been microfabricated which, when the interior surfaces of the first and second planar halves are aligned in facing abutment with each other form elongate bore 508A, 508B. The elongate bore communicates with inlet port 510A, 510B and outlet port 512A, 512B, which enables the downstream passage of fluid from an external source through the elongate bore. In the embodiment illustrated in FIG. 24A, NMR detection compartment 514A around which is NMR rf microcoil 516A is situated downstream from inlet port 510A and in fluid communication with the elongate bore.

Detailed Description Text (131):

In the embodiment illustrated in FIG. 24B, NMR detection compartment 514B, around which is NMR rf microcoil 516B, are housed in module 518B. Elongate bore 508B terminates in upstream and downstream means 520B and 522B to form a liquid-tight, zero dead volume seal with complementary means 524B and 526B in module 518B.

Detailed Description Text (133):

The circuitry illustrated in FIG. 24A comprises means 534A for introducing an NMR signal through a tone/match circuit 536A into microcoil 516A. After passing through microcoil 516A, the signal is fed into preamplifier circuit 538A and to means 538A for receiving the coil output signal.

Detailed Description Text (134):

The circuitry illustrated in FIG. 24B comprises means 534B for introducing an NMR signal into microcoil 516B. Device 500B and module 518B include means 542B, 542B' and 544B, 544B' for establishing electrical communication therebetween. After passing through microcoil 516B, the signal is fed into preamplifier circuit 538B and to means 538B for receiving the coil output signal.

Detailed Description Text (136):

In addition to the NMR rf microcoil detector, other detection means may be interfaced with sample separation compartment, e.g., to serve as a sample detector and NMR trigger. For this purpose, an aperture is formed for communication with the elongate bore or sample processing compartment formed in the support body at a point upstream of the NMR microcoil detection compartment. A second aperture may be formed to communicate with the elongate bore or sample processing compartment downstream of the NMR microcoil detection compartment. The apertures serve as conduits for placing lightguide means, electrodes or other detection means in communication with the elongate bore or sample processing compartment to detect a sample passing therethrough. For example, first and second lightguide means (not shown) can be interfaced with first and second apertures to communicate with the sample processing compartment. Such lightguides can comprise optical fibers that are capable of sample illumination and light collection to enable near IR or UV-VIS optical detection of separated analytes passing through the separation compartment. The detection of separated analytes prior to their entry into the NMR detection chamber may be used for NMR signal enhancement. For example, the flow rate of analyte can be adjusted to increase its concentration within the NMR detection chamber.

Detailed Description Text (137):

The device with rf microcoil and associated fluid inputs and outputs is intended to be placed at the isocenter of the magnetic field of an NMR spectrometer. Various interfacing arrangements can be envisaged.

Detailed Description Text (138):

In one arrangement, as shown in FIG. 25, the separation device shown generally at 500C having an integrated rf microcoil 516C is hyphenated with a miniature (tabletop) NMR magnet shown at 550. The miniature NMR magnet includes tune-and-match circuitry 552, transmit/receive electronics 554, and central computer 556 for acquisition control, data storage and signal processing. Associated electronics 558 for frequency synthesis, amplifiers, waveform generators, attenuators and pulse sequence generators for signal acquisition are controlled by the computer. Thus, in this arrangement, the tune-and-match circuitry 552 as well as the preamplifiers are separate from the miniaturized device and part of the standard configuration of the NMR magnet. Alternatively, device 500C can be fabricated as illustrated in FIG. 24A to include tune-and-match circuitry as well as preamplifiers, and the tune/match/preamplifier circuitry on the miniature NMR magnet can be bypassed.

Detailed Description Text (139):

In a second arrangement, the miniaturized separation device 500A, as illustrated in FIG. 24A, with rf microcoil 516A is hyphenated into a micromagnet NMR system. Recent technological advances in high temperature superconductors, refrigeration and pulsed field magnets suggest the feasibility of much smaller "table-top" magnets. These micro-magnets may be configured to accept the device with rf microcoil having on-board preamplifiers and/or tune and match circuitry in a manner analogous to a CD player accepting a CD disk. This micro-magnet configuration could be used as well to examine multiple devices in series, e.g., fed in on a roll following sample preparation in a "batch" mode, thereby allowing routine automated detection of multiple samples.

Detailed Description Text (140):

The advantages of having a miniaturized separation device integrated with an NMR rf microcoil include: (1) a faster time-to-result with on-line NMR analysis; (2) avoidance of sample dilution from remote sample injection; (3) sample handling capability to provide an NMR-ready sample at the point of detection; (4) increased sensitivity of NMR detection; and (5) low cost-high volume manufacture of consumable devices that are small enough to be accommodated in any high field NMR system.

Detailed Description Text (144):

The device described is a planar column liquid handling device capable of both solid phase sample extraction and structural determination of separated sample constituents by Nuclear Magnetic Resonance ("NMR"). Hyphenated sample processing and detection minimizes dead volume, sample volume required and solvent composition. Time to result is enhanced as a result by use of the device.

Detailed Description Text (145):

FIG. 26A and FIG. 26B illustrate top and side view of planar column device 600 with integrated NMR rf microcoil 602 includes sample and buffer inlet ports 604, 606, 608, 610, in switchable fluid communication via manifold 612 with solid phase extraction chamber 614 by inlet port 614. Extraction chamber 614 is filled with solid phase extraction material 618. The design of the device is based on the multifold geometry disclosed in commonly assigned U.S. Pat. No. 5,658,413 to Kaltenbach et al. for "Miniaturized Planar Columns in Novel Support Media for Liquid Phase Analysis."

Detailed Description Text (146):

Extraction chamber 614 is connected to an outlet channel 620 which, in turn, is connected with rotor 622, e.g., a port valve 624 and conduit 626 assembly, for selectively directing the flow from the outlet channel to waste outlet 628 or to valve/port 630 that leads through the device thus allowing further processing or analysis of the sample. For example, valve 624 would direct voiding of unwanted fluid from solid phase extraction chamber 614 or through-flow to an NMR detection chamber 632. Fluid that is to be flushed from the system/device to waste, for example, would flow from solid-phase extraction chamber 614 through channel 620 to valve/port 624 and into conduit 626 to waste outlet 628, by which fluid can be voided to the exterior of the device. Closing of waste outlet 628, and opening of valve/port 630 allows fluid to continue through channel 634 and, thereafter, through NMR detection chamber 632. Fluid exits detection chamber 632 through port 636, from which the effluent may be collected or discarded.

Detailed Description Text (151):

The following step are the sample elution step. Solutes of increasing hydrophobic character elute according to increasing percentage of organic modifier in the release solution. Rotor 622 is positioned so that valves/ports 624 and 630 are in fluid communication with inlet port 616 and outlet port 636. In five steps ranging from 20% methanol to 100% methanol using 20% increasing increments of methanol, solutes are eluted from the packed bed medium 618 in chamber 614 to the NMR detection chamber 632. At each elution step, the NMR spectrum of the eluted sample is obtained.

Detailed Description Text (153):

The NMR parameters of a pair of molecules related as object to mirror image are identical; chiral discrimination depends on creation of a diastereoisomeric entities. Since spectral differences between the antipodal pair can be realized, NMR determination of chiral excess is superior to optical methods which only yield the sum total contributions from dextro- and levo- forms. The efficiency of chiral analysis by NMR depends on the ability to yield well-resolved resonances for each member of the antipodal pair and, although NMR is comparatively less sensitive than LC, detection of 1% (w/w) is possible.

Detailed Description Text (154):

Strategies for chiral discrimination by NMR include derivatization methods, in which covalent linkages are formed between the analyte and the reagent molecule, or formation of diastereoisomeric compounds held together by weak physical forces, e.g., ionic, dipolar, hydrogen bonding, and pi-pi interactions. General classes of chiral agents include chiral solvating agents, excluding metal chelates, chiral derivatizing agents, which form separate derivatives through, e.g., formation of a covalent bond, and paramagnetic shift reagents, that result in differential binding

to the solute enantiomers and perturbing chemical shift due to the preferentially bound enantiomer. Examples of compounds providing chiral discrimination include cyclodextrins, acidic reagents such as mandelic acid, chiral lanthanide shift reagents such as tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium (III), known as Eu(FOD), and esters of O-arylated(R)-lactic acids. In this manner, not only are the spectra of the various drug metabolites present in the urine sample obtained, but enantiomeric excess can also be determined.

Detailed Description Text (155):

Plug flow of the released solute arrived at the NMR chamber via the one-port, two-position rotor 622. Sample detection can either be through stopped flow or flow-through mode. Stopped flow involves acquisition of the NMR signal while the sample plug resides in the homogeneous volume of the detector coil. The region of signal is roughly defined as the volume of the sample enclosed by the radio-frequency coil 602. There is little signal detection from the edges of the coil. With the stopped flow method, signal averaging continues until enough signal is obtained from the sample, minimally 3:1 signal-to-noise. With the flow-through method, signal will be obtained from sample from the time it enters the coil 602, until no more sample is released from the solid phase extraction chamber 614. Some contamination of signal may arise when acquisition occurs before sample arrives in the homogeneous volume of the coil and after it leaves the homogeneous volume of the coil. Volume localized spectroscopy has been used to determine how much of a sample is contributing to a signal within the homogeneous volume. Spectra can be summed and weighted depending on the ratio of sample to unwanted signal.

CLAIMS:

1. A miniaturized total analysis system for liquid phase sample preparation and detection comprising:

a microfabricated support body having first and second substantially planar opposing surfaces wherein the support body has a microchannel microfabricated in the first planar surface;

a cover plate arranged over the first planar surface, wherein the cover plate in combination with the first microchannel forms a sample processing compartment;

an inlet port and an outlet port communicating with the sample processing compartment, wherein the inlet and outlet ports enable downstream passage of fluid from an external source through the sample processing compartment; and

downstream from the sample processing compartment and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil, wherein the NMR detection compartment and the NMR rf microcoil are housed within the support body.

2. The miniaturized total analysis system of claim 1, wherein the NMR detection compartment and the NMR rf microcoil comprise microstructures fabricated in the support body.

3. The miniaturized total analysis system of claim 1, wherein the NMR detection compartment and the NMR rf microcoil comprise a modular structure removably insertable into the support body.

4. The miniaturized total analysis system of claim 1, wherein the NMR rf microcoil is selected from the group consisting of solenoid coils, Helmholtz coils, surface coils and birdcage coils.

6. The miniaturized total analysis system of claim 4, wherein the NMR rf microcoil is a receive-only coil.

7. The miniaturized total analysis system of claim 6, wherein the NMR rf microcoil is comprised of multiple receive-only coils.

9. The miniaturized total analysis system of claim 1, wherein the NMR detection compartment has a volume of approximately 5 nl to approximately 10 .mu.l.

17. A miniaturized total analysis system for liquid phase sample preparation and

detection, comprising:

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces;

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other;

an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore;

an inlet port and an outlet port communicating with the elongate bore, the ports enabling the downstream passage of fluid from an external source through the elongate bore; and

downstream from the elongate bore and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil, wherein the NMR detection compartment and the NMR rf microcoil are housed within the support body.

18. The miniaturized total analysis system of claim 17, wherein the NMR detection compartment and the NMR rf microcoil comprise microstructures fabricated in the support body.

19. The miniaturized total analysis system of claim 17, wherein the NMR detection compartment and the NMR rf microcoil comprise a modular structure removably insertable into the support body.

20. The miniaturized total analysis system of claim 17, wherein the NMR rf microcoil is selected from the group consisting of solenoid coils, Helmholtz coils, surface coils and birdcage coils.

22. The miniaturized total analysis system of claim 20, wherein the NMR rf microcoil is a receive-only coil.

23. The miniaturized total analysis system of claim 22, wherein the NMR rf microcoil is comprised of multiple receive-only coils.

25. The miniaturized total analysis system of claim 17, wherein the NMR detection compartment has a volume of approximately 5 nl to approximately 10 .mu.l.

33. A miniaturized total analysis system for liquid phase sample preparation and detection, comprising:

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces;

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other;

a sample processing compartment comprising an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore;

an inlet port and an outlet port communicating with the sample processing compartment, the ports enabling the downstream passage of fluid from an external source through the sample processing compartment; and

downstream from the sample processing compartment and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil, wherein the NMR detection compartment and the NMR rf microcoil are housed within the support body.

34. The miniaturized total analysis system of claim 33, wherein the NMR detection compartment and the NMR rf microcoil comprise microstructures fabricated in the

support body.

35. The miniaturized total analysis system of claim 33, wherein the NMR detection compartment and the NMR rf microcoil comprise a modular structure removably insertable into the support body.

36. The miniaturized total analysis system of claim 33, wherein the NMR rf microcoil is selected from the group consisting of solenoid coils, Helmholtz coils, surface coils and birdcage coils.

38. The miniaturized total analysis system of claim 36, wherein the NMR rf microcoil is a receive-only coil.

39. The miniaturized total analysis system of claim 38, wherein the NMR rf microcoil is comprised of multiple receive-only coils.

41. The miniaturized total analysis system of claim 33, wherein the NMR detection compartment has a volume of approximately 5 nl to approximately 10 .mu.l.

49. An integrated device for sample preparation and NMR detection, comprising:

(a) a miniaturized total analysis system for liquid phase sample preparation and detection, comprising

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces,

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other,

an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore,

an inlet port and an outlet port communicating with the elongate bore, the ports enabling the downstream passage of fluid from an external source through the elongated bore

downstream from the elongate bore and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil, wherein the NMR detection compartment and the NMR rf microcoil are housed within the support body; and

(b) a magnet configured to accept the miniaturized total analysis system, wherein the device is capable of generating an NMR spectrum.

50. The integrated device of claim 49, wherein the NMR detection compartment and the NMR rf microcoil comprise microstructures fabricated in the support body.

51. The integrated device of claim 49, wherein the NMR detection compartment and the NMR rf microcoil comprise a modular structure removably insertable into the support body.

52. The integrated device of claim 49, wherein the NMR rf microcoil is selected from the group consisting of solenoid coils, Helmholtz coils, surface coils and birdcage coils.

54. The integrated device of claim 52, wherein the NMR rf microcoil is a receive-only coil.

55. The integrated device of claim 54, wherein the NMR rf microcoil is comprised of multiple receive-only coils.

57. The integrated device of claim 49, wherein the NMR detection compartment has a volume of approximately 5 nl to approximately 10 .mu.l.

65. An integrated device for sample preparation and NMR detection, comprising:

(a) a miniaturized total analysis system for liquid phase sample preparation and detection, comprising

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces,

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other,

a sample processing compartment comprising an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore,

an inlet port and an outlet port communicating with the sample processing compartment, the ports enabling the downstream passage of fluid from an external source through the sample processing compartment, and

downstream from the sample processing compartment and in fluid communication therewith, an NMR detection compartment around which is an NMR rf microcoil, wherein the NMR detection compartment and the NMR rf microcoil are housed within the support body; and

(b) a magnet configured to accept the miniaturized total analysis system, wherein the device is capable of generating an NMR spectrum.

66. The integrated device of claim 65, wherein the NMR detection compartment and the NMR rf microcoil comprise microstructures fabricated in the support body.

67. The integrated device of claim 65, wherein the NMR detection compartment and the NMR rf microcoil comprise a modular structure removably insertable into the support body.

68. The integrated device of claim 65, wherein the NMR rf microcoil is selected from the group consisting of solenoid coils, Helmholtz coils, surface coils and birdcage coils.

70. The integrated device of claim 68, wherein the NMR rf microcoil is a receive-only coil.

71. The integrated device of claim 70, wherein the NMR rf microcoil is comprised of multiple receive-only coils.

73. The integrated device of claim 68, wherein the NMR detection compartment has a volume of approximately 5 nl to approximately 10 .mu.l.

86. A miniaturized total analysis system for liquid phase sample preparation and detection comprising:

a microfabricated support body having first and second substantially planar opposing surfaces wherein the support body has a microchannel microfabricated in the first planar surface;

a cover plate arranged over the first planar surface, wherein the cover plate in combination with the first microchannel forms a sample processing compartment;

an inlet port and an outlet port communicating with the sample processing compartment, wherein the inlet and outlet ports enable downstream passage of fluid from an external source through the sample processing compartment; and

downstream from the sample processing compartment, a module comprising an NMR detection compartment and an NMR rf microcoil, the module being insertable into the support body such that the NMR detection compartment is in fluid communication with the sample processing compartment.

87. A miniaturized total analysis system for liquid phase sample preparation and detection, comprising:

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces;

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other;

an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore;

an inlet port and an outlet port communicating with the elongate bore, the ports enabling the downstream passage of fluid from an external source through the elongate bore; and

downstream from the elongate bore, a module comprising an NMR detection compartment and an NMR rf microcoil, the module being insertable into the support body such that the NMR detection compartment is in fluid communication with the elongate bore.

88. A miniaturized total analysis system for liquid phase sample preparation and detection, comprising:

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces;

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other;

a sample processing compartment comprising an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore;

an inlet port and an outlet port communicating with the sample processing compartment, the ports enabling the downstream passage of fluid from an external source through the sample processing compartment; and

downstream from the sample processing compartment, a module comprising an NMR detection compartment and an NMR rf microcoil, the module being insertable into the support body such that the NMR detection compartment is in fluid communication with the sample processing compartment.

89. An integrated device for sample preparation and NMR detection, comprising:

(a) a miniaturized total analysis system for liquid phase sample preparation and detection, comprising:

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces;

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other;

an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore;

an inlet port and an outlet port communicating with the elongate bore, the ports enabling the downstream passage of fluid from an external source through the elongate bore; and

downstream from the elongate bore, a module comprising an NMR detection compartment and an NMR rf microcoil, the module being insertable into the support body such that the NMR detection compartment is in fluid communication with the elongate bore; and

(b) a magnet configured to accept the miniaturized total analysis system, wherein the device is capable of generating an NMR spectrum.

90. An integrated device for sample preparation and NMR detection, comprising:

(a) a miniaturized total analysis system for liquid phase sample preparation and detection, comprising:

a microfabricated support body having first and second component halves each having substantially planar opposing interior and exterior surfaces;

a first microchannel microfabricated in the interior surface of the first support body half and a second microchannel microfabricated in the interior surface of the second support body half, wherein each of the microchannels is so arranged as to provide the mirror image of the other;

a sample processing compartment comprising an elongate bore formed by aligning the interior surfaces of the support body halves in facing abutment with each other whereby the microchannels define the elongate bore;

an inlet port and an outlet port communicating with the sample processing compartment, the ports enabling the downstream passage of fluid from an external source through the sample processing compartment; and

downstream from the sample processing compartment, a module comprising an NMR detection compartment and an NMR rf microcoil, the module being insertable into the support body such that the NMR detection compartment is in fluid communication with the sample processing compartment; and

(b) a magnet configured to accept the miniaturized total analysis system, wherein the device is capable of generating an NMR spectrum.